EDUCATING PATIENTS: COMMUNICATING THE GUT-BRAIN CONNECTION IN PARKINSON'S DISEASE USING MULTIMEDIA

by
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Abstract

Parkinson's disease (PD) is a type of synucleinopathy that is characterized by abnormal accumulation of α-synuclein (α-syn) aggregates in neurons (Challis et al. 2020). The misfolded α-syn triggers self-aggregation into neurotoxic amyloid fibrils that can be transmitted from cell to cell, ultimately causing neurodegeneration (Kim et al. 2019). Mounting evidence suggests that for a majority of PD patients, this process can originate in the gut and ascend to the brain via the vagus nerve (Kim et al. 2019). Once in the brain, α-syn pathology propagates to reach the midbrain leading to motor dysfunction.

Since PD is mostly known as a motor disorder, its non-motor symptoms often go undetected. By the time patients begin exhibiting motor symptoms, the disease is usually advanced to the extent that only symptoms can be treated (Dawson et al. 2019). Recent studies suggest that non-motor symptoms, particularly those associated with gastrointestinal (GI) dysfunction may appear as many as 20 years prior to neurological symptoms (Challis et al. 2020). It is important for the public, especially those with chronic GI complications to understand how prolonged GI symptoms may indicate early-stage PD.

However, two factors currently hinder education in this area: (i) scientific research papers are difficult to understand particularly for readers without a scientific background; and (ii) current visual resources for this topic are limited to schematic, often confusing diagrams and inaccurate anatomical and molecular images. In particular, spatial relationships between the gut and the brain are

difficult to depict in 2D. And, no effective didactic 3D visualizations exist to address this important public health topic.

I propose an interactive platform of (i) a foundational 2D teaching module on the scientific background and pathogenesis of PD, and (ii) a narrative 3D animation highlighting recent studies of the gut-brain connection in PD. Learners will navigate at their own pace through the introductory learning module prior to viewing the more complex material.

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Introduction

Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by progressive neurodegeneration not only of the dopaminergic nigrostriatal system, responsible for the core motor symptoms, but also by involvement of other neuronal systems and organs responsible for the non-motor symptoms (Jellinger 2011). PD is associated with widespread occurrence of intracytoplasmic inclusions, Lewy bodies (LBs) and Lewy neurites (LNs), due to deposition of phosphorylated α-synuclein (α-syn), the major protein marker and biological hallmark of PD (Spillantini et al. 1997). The disease process is multifocal and involves α-syn accumulation in the central nervous system neurons as well as peripheral autonomic nervous system neurons (Braak et al. 2009).

In 2003, a staging scheme for idiopathic PD was introduced, according to which α -syn pathology originates in the dorsal motor nucleus of the vagus (DMV) and progresses from there to other brain regions, including the substantia nigra (SN), a midbrain dopaminergic nucleus which plays a critical role in motor control (Sonne et al. 2020). However, analysis of human pathology led to the Braak hypothesis which suggests that α -syn pathology could originate in the gut instead of the DMV and spread to the brain via the vagus nerve (Kim et al. 2019). Further studies on the gut-brain connection in PD has led to stronger support for this hypothesis and suggested directions for potential therapeutic opportunities (Dawson et al. 2019).

Current Research on the Gut-Brain Connection in PD

i. Alpha-synucleinopathy in the Gut

Alpha-synucleinopathies are characterized by aggregation of misfolded α-syn proteins, often resulting in symptoms associated with PD. Growing evidence suggests that exposure to microbial amyloids in the gastrointestinal (GI) tract may trigger α-syn misfolding and aggregation in the periphery (Sampson et al. 2020). For example, Enterobacteriaceae, such as *E. coli*, are highly prevalent within the gut microbiome in humans. They abundantly express the cell surface amyloid proteins known as curli fibers, which are capable of transiently interacting with α-syn and accelerating its misfolding and aggregation in a prion-like manner (Sampson et al. 2020). Colonization of human α-syn with curli-producing *E. coli* has shown to induce accumulation of α-syn aggregates at GI tract before spreading to the brain. Furthermore, increased colonization with *E. coli* has been reported in individuals with PD compared to healthy controls, supporting the association of gut bacterial amyloid exposure with PD pathology (Sampson et al. 2016).

ii. Transneuronal Propagation of Pathologic α-syn via Vagus Nerve

The Braak hypothesis outlining the gut-to-brain spread of α-syn pathology via the vagus nerve was tested by recent studies using a mouse model. The studies involved injecting pathological α-syn preformed fibrils (PFF) into the duodenal and pyloric muscularis layer and assessing spread of pathologic α-syn in brain (Kim et al. 2019). Following the gut injection of α-syn PFFs, spread of pathologic α-syn was observed in the brain in a pattern corresponding to Braak's staging scheme for

idiopathic PD: from olfactory bulb and/or DMV to medulla and pontine tegmentum, amygdala and substantia nigra, temporal cortex, then to neocortex (Braak et al. 2009). Furthermore, the enteric nervous system (ENS) such as the myenteric plexus of the pylorus and duodenum demonstrated pathologic α-syn in enteric neurons, denoting that the α-syn PFF were templating the endogenous α-syn to misfold (Kim et al. 2019). This finding supports the cell-to-cell transmission of pathologic α-syn in a prion-like manner. In addition, to evaluate if pathologic α-syn is transmitted to the brain via the vagus nerve, truncal vagotomy was conducted after α-syn PFF injection in the gut (Kim et al. 2019). Cutting the vagus nerve prevented the gut-to-brain spread of α-synucleinopathy and PD-associated symptoms, supporting the idea that the vagus nerve is the key route for the transneuronal propagation of pathologic α-syn (Kim et al. 2019).

iii. Potential Therapeutic Opportunities

Although there is no cure for PD to date, advanced studies in the gut-brain connection in PD have opened the door to multiple therapeutic targets. Aside from targeting the genetic causes of PD, there have been clinical trials using monoclonal antibodies against α-syn to reduce its concentration and to prevent transneuronal propagation of pathologic α-syn (Dawson et al. 2019). Because the treatment would be optimally effective when initiated during the early stages of PD (Dawson et al. 2019), it is more important than ever to encourage patients to participate in clinical trials before further progression of PD.

Existing Visual Resources

The anatomy of ENS is complex and difficult to visualize as there are limited visual resources, such as schematic 2D diagrams and fluoroscopy images. Many existing resources depict inaccurate scale of enteric neurons relative to the surrounding structures such as intestinal villi and vagus nerve. The spatial anatomy of the ENS in relation to the intestinal villi and vagus nerve is important in understanding the gut-brain axis and the pathology of PD. Yet, no effective didactic 3D visualizations exist to address this important public health topic.

Effectiveness of Multimedia as a Teaching Tool

i. Interactive Website

Compared to non-interactive teaching material, interactive media has been shown to accelerate teaching and learning processes (Maaruf et al. 2013). Various studies have compared the effectiveness of interactive teaching material and traditional teaching material by testing two samples of students on their academic performance after being taught using each method. Many studies have concluded that student performance was greatly improved when an interactive teaching technique was applied as compared to traditional classroom (Ilhan et al. 2016). The studies revealed that students find the interactive teaching model useful because it increased their level of understanding of the material, long-term memory, and learning satisfaction (Abdulrahaman et al. 2020).

ii. 3D Animation

In a study that tested the effectiveness of 3D digital animation in teaching human anatomy, the resulting data supported that 3D animations were effective in teaching human anatomy especially in recalling anatomical knowledge requiring spatial ability, as compared to sole use of 2D images (Hoyek et al. 2014). Other studies have also shown higher learning satisfaction and engagement as well as academic performance when 3D animation was used instead of 2D media (Triepels et al. 2019).

Cognitive Theory of Multimedia Learning

Cognitive Theory of Multimedia Learning proposed by Mayer (2009) presents the idea that "the brain does not interpret a multimedia presentation of words, pictures, and auditory information in a mutually exclusive fashion; rather these elements are selected and organized dynamically to produce logical mental constructs." His theory is based on three main assumptions:

1. *The Dual Channel Assumption*: The human brain has two separate channels (auditory and visual) for processing information;
2. *The Limited Capacity Assumption*: Each channel has limited space for information processing;
3. *The Active Processing Assumption*: Learning is an active process of filtering, selecting, organizing, and integrating information based upon prior knowledge.

In his third assumption, Mayer highlights the importance of learning when new information is integrated with prior knowledge. These findings will be incorporated in this project, which will be discussed in detail in the *Materials and Methods* section.

Intended Audience: Challenges and Goals

The primary audience for this project is PD patients and their families, and the lay public. The secondary audience includes researchers investigating treatments for PD, as well as philanthropists interested in supporting further research. All material is geared towards an audience with high-school level scientific knowledge.

Several challenges exist in creating an educational material for the PD patients and their families: (i) PD can affect the patient's eyesight over time and the ability to focus in situations that divide attention (Goldman et al. 2018); (ii) the topic of gut-brain connection in PD is complex to understand without scientific knowledge; and (iii) requires the audience to have background neurophysiologic information about PD. To overcome these challenges, the project must aim for the fine balance between the extent of foundational information and teaching effectiveness.

Project Objectives

The objectives of this thesis project are to:

1. Educate the audience by designing a resource that allows interactive exploration into the gut-brain connection in Parkinson's disease, from basic to complex. This resource will: (i) introduce the audience to the scientific concept and background neurophysiologic information, (ii) provide a general overview of the pathogenesis, and (iii) allow the viewer to integrate this information to understand the most recent scientific studies and advances.
2. Provide educational visual material that is easily accessible and understandable by the public.
3. Promote public awareness of the early signs of PD and encourage individuals to seek appropriate care to prevent further progression of the disease.
4. Encourage patients to participate in clinical trials of early-stage treatments for PD (Dawson et al. 2019).

Potential Contribution to Public Health and Biocommunication

With the rapid growth of new research and increased awareness and public interest in PD, there is a critical need for biocommunicators to bridge the gap between the scientific community and the lay audience. Through the use of various media (e.g. interactive website, 2D images and animations, and 3D animation), I will provide a novel approach to patient education about PD.

Materials and Methods

Content Research

In addition to an extensive literature search, additional published papers and data were provided by The Dawson Lab in The Johns Hopkins Institute for Cell Engineering. Each paper was thoroughly studied and summarized to compile a pool of references. For easy access in the future, 40 papers in total were categorized and organized as follows:

a. Braak hypothesis and Lewy pathology
b. General pathology of Parkinson's Disease
c. Gut-to-brain transmission of pathologic α-syn
d. Gut microbiota as a cause of α-syn misfolding
e. ENS and enteric neuron structure
f. α-syn structure
g. α-syn receptor (LAG-3)

Regular meetings were held with preceptors for additional discussion and to verify accuracy of the content. One challenge during the literature review was that many mechanisms are yet unknown since research on this topic is currently ongoing. This will be further addressed in the *Discussion* section.

Project Planning and Design

i. Interactive Website: Flowchart and Wireframe

Prior to creating the interactive website, a flowchart (**Figure 1**) was constructed using simple diagrams for informational hierarchy. Each page of the

website was planned with regards to its content and media type (screen, interactive, or animation).

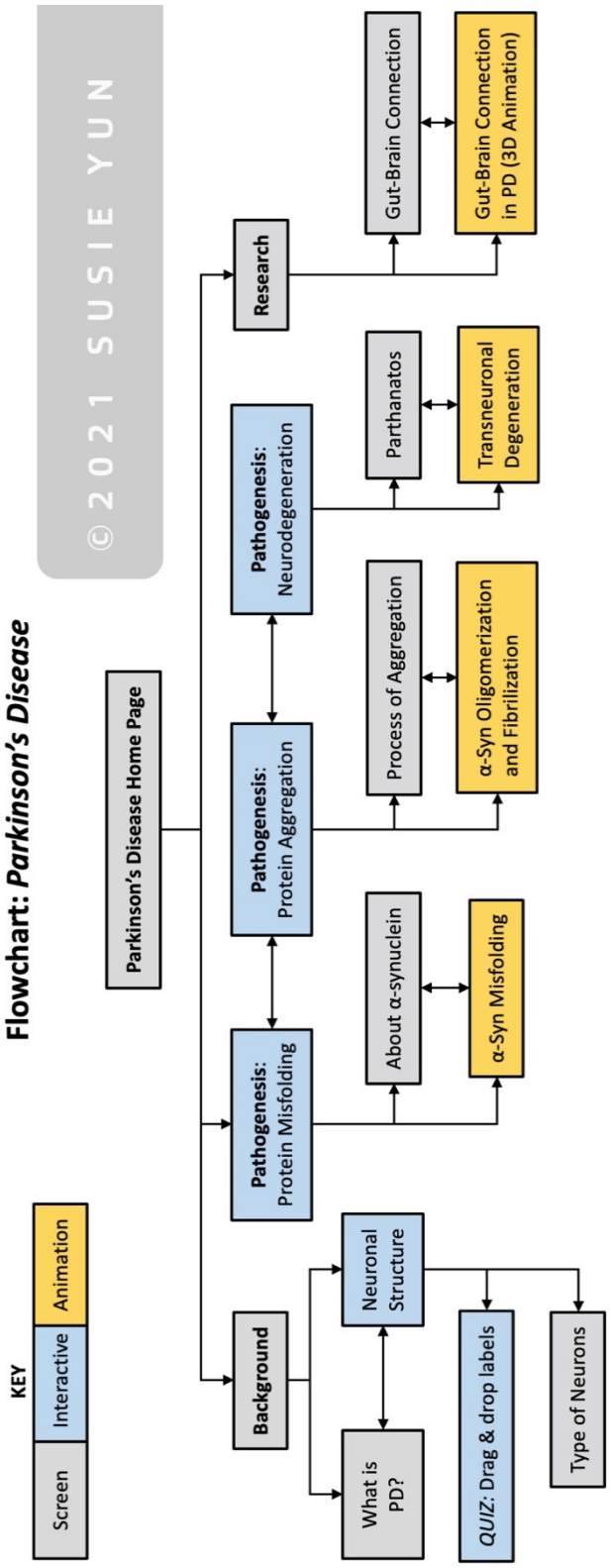


Figure 1. Flowchart of the interactive website. The type of media (screen, interactive, of animation) for each page is indicated by different colors (see *Key*).

Following the flowchart, a wireframe (**Figure 2**) was created using Adobe XD to visualize the framework of the website. The design and placement of each visual element were decided at this stage emphasizing an effective user interface and user experience.

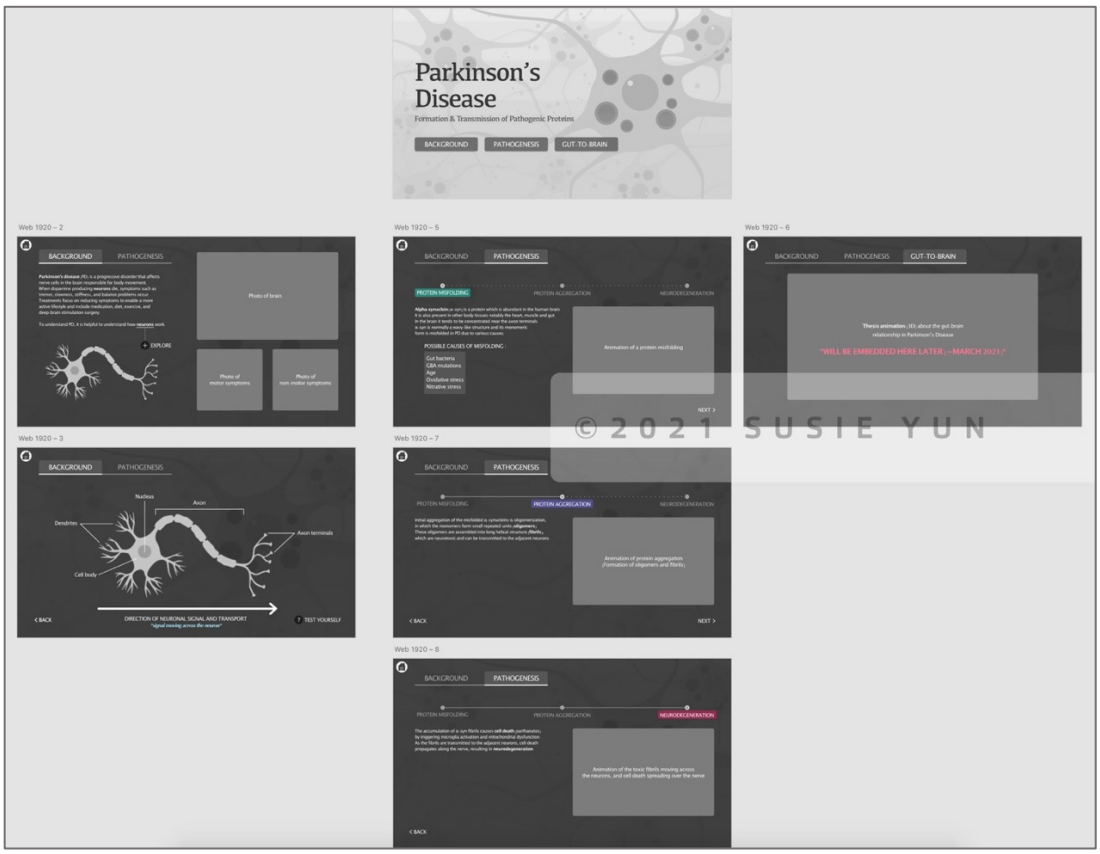


Figure 2. Wireframe of the interactive website. Not all pages were included in the wireframe due to redundancy. Not all of the text intended to be read.

ii. 3D Animation: Story Outline

Based on the summaries of existing literature documented during *Content Research*, a preliminary story outline (**Figure 3**) was created for content breakdown as well as script planning. Multiple revisions with the project preceptors and advisor resulted in the editing of some content. Additional information was added in several areas to improve clarity.

1. Introduction
    a. What is PD? – general description then about movement disorder
    b. Motor and non-motor symptoms
        i. **Motor**: bradykinesia (slowness of movement), rigidity, rest tremor, gait disturbance (postural instability)
        ii. **Non-motor**: gastrointestinal dysfunction, cognitive dysfunction, autonomic dysregulation, olfactory dysfunction, fatigue, psychosis, sleep disturbances, dementia, and mood disorders
        iii. *"One of the most common non-motor symptoms observed in PD is GI dysfunction, which often precedes the development of motor symptoms."*
    c. Early GI symptoms (eg. constipation, bloating, abdominal pain) in PD patients – why?!
        ~~i. *"GI symptoms occur in the majority of PD patients and constipation is among the most common early symptoms."*~~
    d. PD has been known to begin in the brain, but current (?) research suggests it may actually originate in the GI tract!
2. **Gut in a body: show connection to the brain via vagus n.** (big picture; no details yet)
3. Gut layers with plexuses (Meissner's at submucosa & Auerbach's at muscularis propria)
    a. List the causes of misfolding (GBA mutations, age, nitrative oxidative stress…)
    b. Gut bacteria & "leaky gut" (*"One hypothesis is that…"*)

Formation of pathologic a-syn fibrils

4. Neuron (show whole structure – dendrites, cell body, axon, axon terminal)
    a. EXACTLY WHICH NEURON? – the most distal (closest to the lumen) enteric mucosal neuron? Then pathologic a-syn propagates to terminal of vagus later?
5. **Native monomeric a-syn**
6. **Misfolded monomeric a-syn**
    ~~a. List possible causes (Gut bacteria *leaky gut*, GBA mutations, age, nitrative oxidative stress, cell-to-cell transmission)~~
7. **Oligomers**
8. **Fibrils* → these themselves are toxic to neurons and cause cell death (parthanatos)**
    a. Triggers microglia activation (→ astrocyte activation) & mitochondrial dysfunction
9. **Aggregation into 'pathologic a-syn aggregates'** (→ not necessarily dying due to this; aggregation is a protective mechanism to reduce the [fibrils] in the neuron; LB/LNs are simply the pathology that remains and gets detected in PD patients)
10. Cell death **(Parthanatos)** *not in voice-over/script, but visually depicted in animation*

Transneuronal transmission of pathologic a-syn

11. **Go back to #8 view***
12. Transmission of fibrils to the adjacent neurons (via receptor-mediated endocytosis – LAG3 receptor)
13. Pathologic α-syn seeding (misfolding and aggregation – repeat #5-8)
    a. *"These transmitted α-syn fibrils can act as a **template** for misfolding and aggregation…" forming a template that seeds misfolding for nearby alpha-synuclein protein, turning the previously healthy protein into a pathogenic protein*

~~14. Aggregation into Lewy bodies (and neurites)~~
15. Cell death

Retrograde propagation to the brain

16. Repeat #12-14 in nearby neurons ("chain reaction")
17. Along the vagus n. (camera following upwards, like on a highway/rollercoaster)
18. Reach brain (dorsal motor nucleus of vagus n.)
19. PD symptoms mentioned in #1
20. **Go back to #2 view** (re-emphasize the gut-brain connection)

Figure 3. Preliminary story outline of the 3D animation. This figure shows deletion of unnecessary content in strikethrough and addition of new content in gray highlight.

iii. Software Overview

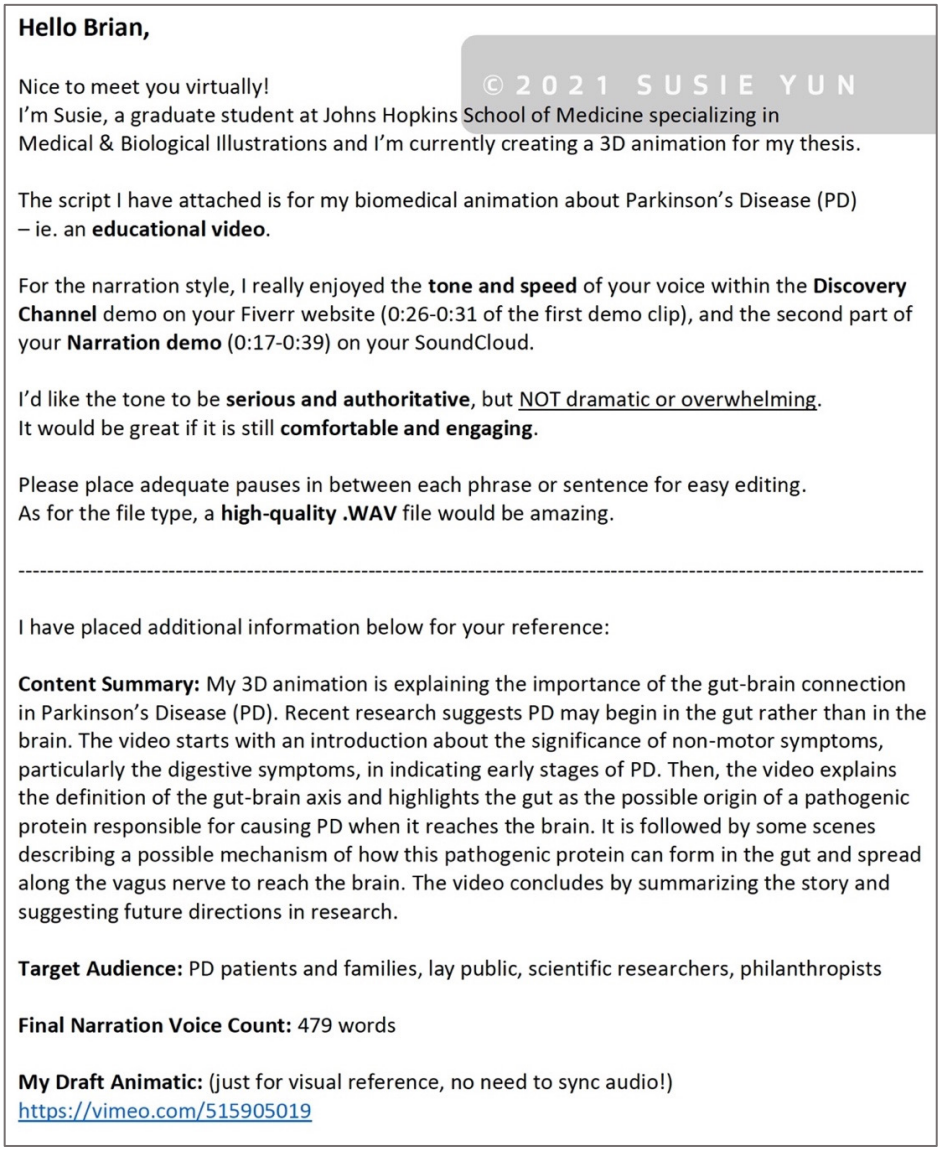
A wide range of software was used to create the interactive website and 3D animation. For the interactive website, Adobe XD was used to create the wireframe and simulate page navigation within the program. Both Adobe Illustrator and Adobe Animate were used to create graphic assets, which were animated and coded in Adobe Animate.

For the 3D animation, Adobe Photoshop and Adobe Illustrator were used to create storyboards. Adobe After Effects was used to animate the final storyboard images into a preliminary animation (animatic), as well as for compositing the final renders created with Maxon Cinema 4D and OctaneRender. For creating and sculpting models with complex geometry, Pixologic Zbrush was used instead of Maxon Cinema 4D. Cinema 4D R23 was used to create, modify, and animate the 3D models. OctaneRender, a third-party unbiased rendering engine, was used for lighting, creating materials, previewing renders in real-time, and rendering the final output with high efficiency.

Script and Narration Development

Following the story outline created earlier, a script was developed using plain language to be easily understood by PD patients and the lay public. In instances where the use of a medical or scientific term was necessary, it was accompanied by its a definition or additional explanation to aid understanding. The script was written as concisely as possible and broad terms were used to describe gaps in current knowledge. General overviews were provided rather than

presenting an exhaustive list of hypotheses and mechanisms. To improve the content accuracy and story flow, the script was reviewed by the preceptor and advisor throughout the process until the voiceover was recorded. The finalized script (**Appendix A**) was narrated and recorded by a professional voice-over artist. Directions for the desired tone and style of the narration were included with the script that was delivered to the voice-over artist, as shown in **Figure 4**.



Hello Brian,

Nice to meet you virtually!
I'm Susie, a graduate student at Johns Hopkins School of Medicine specializing in Medical & Biological Illustrations and I'm currently creating a 3D animation for my thesis.

The script I have attached is for my biomedical animation about Parkinson's Disease (PD) – ie. an **educational video**.

For the narration style, I really enjoyed the **tone and speed** of your voice within the **Discovery Channel** demo on your Fiverr website (0:26-0:31 of the first demo clip), and the second part of your **Narration demo** (0:17-0:39) on your SoundCloud.

I'd like the tone to be **serious and authoritative**, but NOT dramatic or overwhelming.
It would be great if it is still **comfortable and engaging**.

Please place adequate pauses in between each phrase or sentence for easy editing.
As for the file type, a **high-quality .WAV** file would be amazing.

I have placed additional information below for your reference:

Content Summary: My 3D animation is explaining the importance of the gut-brain connection in Parkinson's Disease (PD). Recent research suggests PD may begin in the gut rather than in the brain. The video starts with an introduction about the significance of non-motor symptoms, particularly the digestive symptoms, in indicating early stages of PD. Then, the video explains the definition of the gut-brain axis and highlights the gut as the possible origin of a pathogenic protein responsible for causing PD when it reaches the brain. It is followed by some scenes describing a possible mechanism of how this pathogenic protein can form in the gut and spread along the vagus nerve to reach the brain. The video concludes by summarizing the story and suggesting future directions in research.

Target Audience: PD patients and families, lay public, scientific researchers, philanthropists

Final Narration Voice Count: 479 words

My Draft Animatic: (just for visual reference, no need to sync audio!)
https://vimeo.com/515905019

Figure 4. First page of the voiceover request document. This figure shows the script including directions for tone and style of the narration supplied to the voiceover artist.

In addition, the script was subdivided by scenes and sentences. Further guidance, such as emphasis on keywords, addition of pauses, pronunciation and desired pace was provided for the voiceover artist (**Figure 5**).

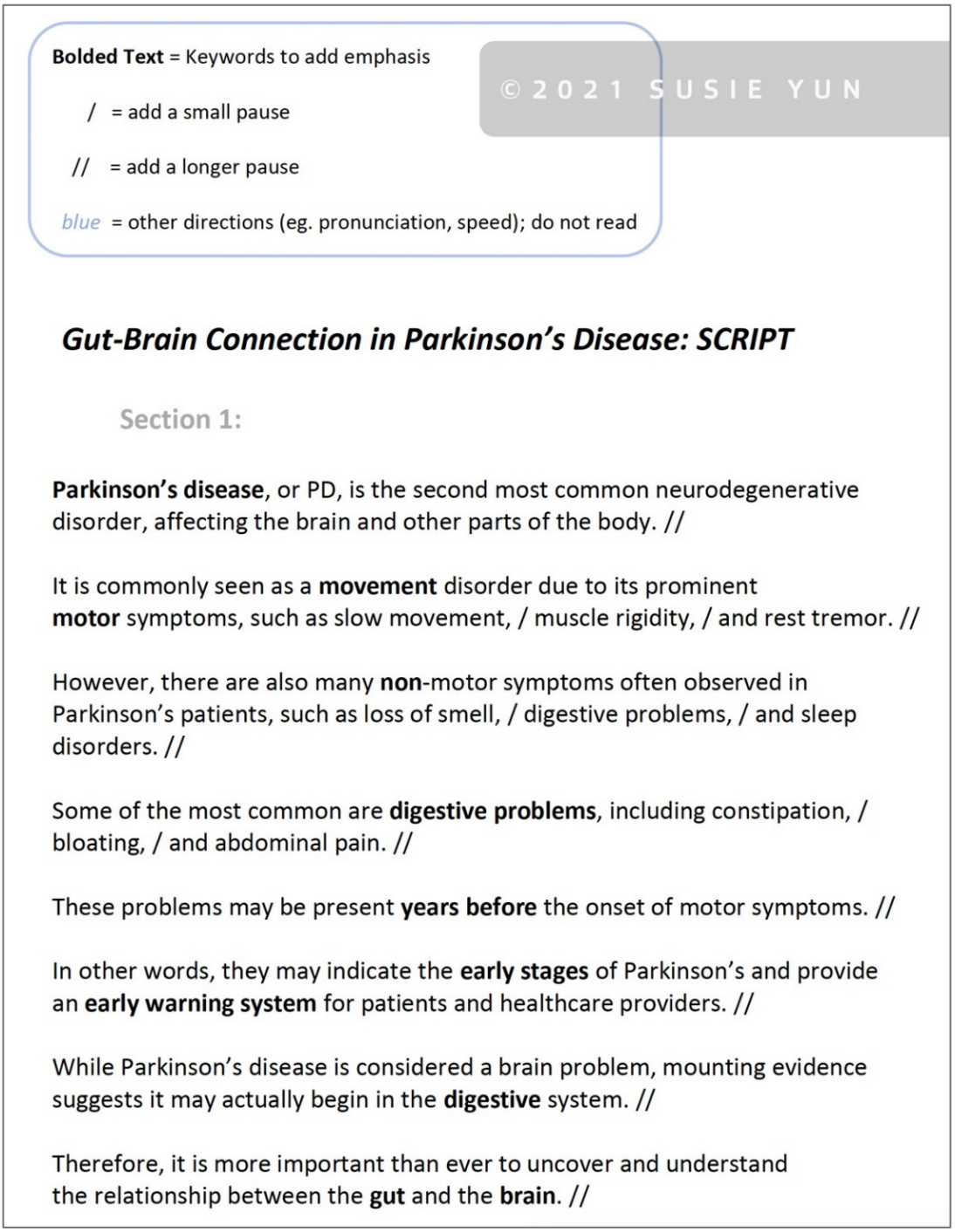


Figure 5. Example of script with further guidance for the voiceover artist. This figure shows the specific keyword emphasis and pacing requested.

Storyboard Development

i. Interactive Website

Prior to creating the 2D animation included in the interactive website, a storyboard was developed for effective planning (**Figure 6**). For each animation which was less than 10 seconds long, 3 to 5 images were created.

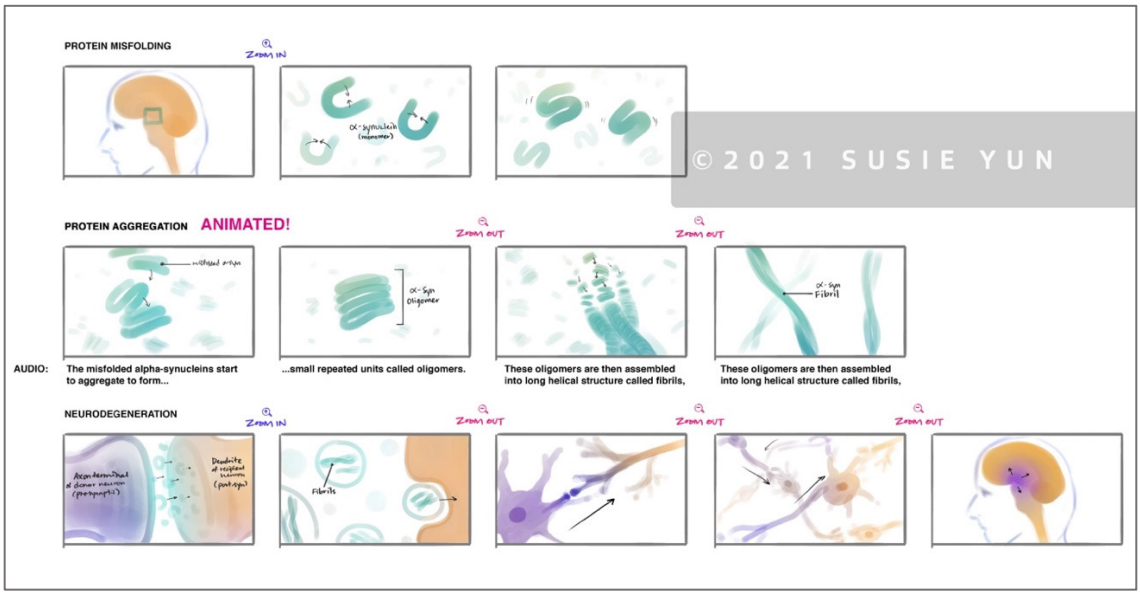


Figure 6. Storyboard for the interactive website. Only the second storyboard, *Protein Aggregation*, was animated as an example for this project, which will be further discussed later in the *Results* section. Text not intended to be read.

ii. 3D Animation

Prior to storyboard development, the final script was broken down by scenes and shots, which were accompanied by rough thumbnail sketches to brainstorm initial ideas (**Figure 7**).

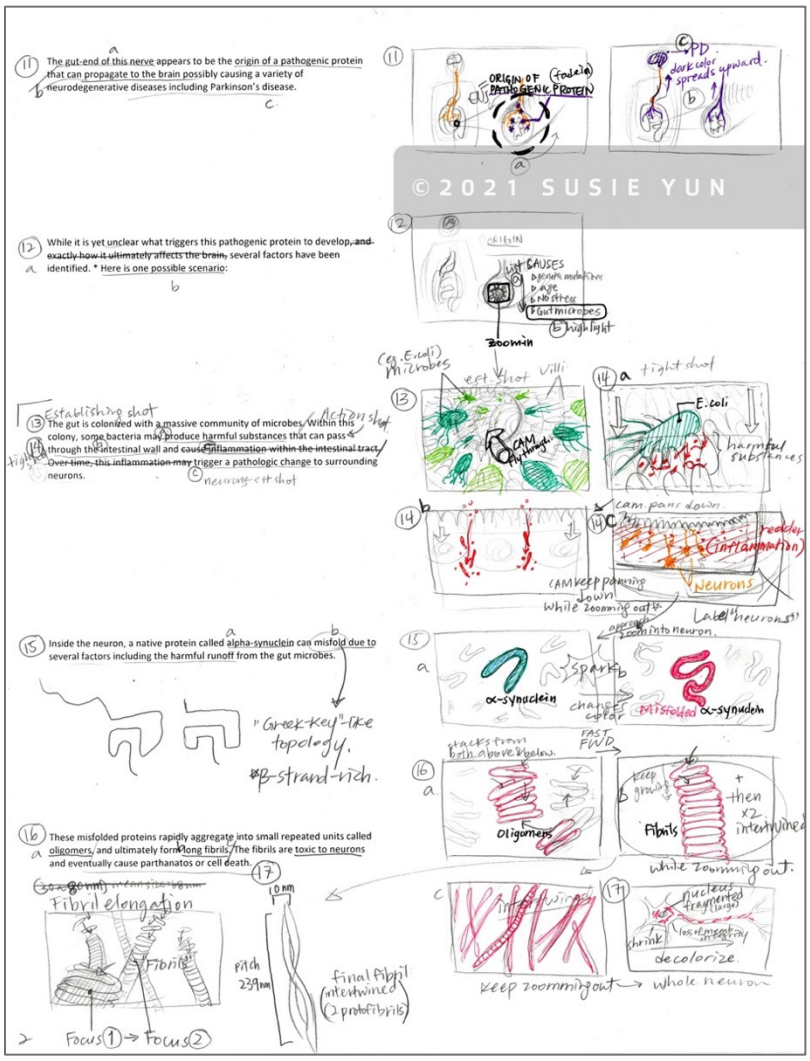


Figure 7. Thumbnail sketches for the 3D animation storyboard. This figure shows initial ideas based on the script. Text not intended to be read.

Following the thumbnail sketches, an initial storyboard was developed digitally using Adobe Photoshop and Adobe Illustrator. Some images were created with colors where needed, such as to emphasize or distinguish certain elements. For example, the normal α-syn was represented in green and the misfolded α-syn in red to follow the color convention widely accepted by the researchers studying this topic. Through several meetings and revisions with preceptors and advisor, 6 iterations were produced to create the final storyboard (**Figure 8; Appendix B**).

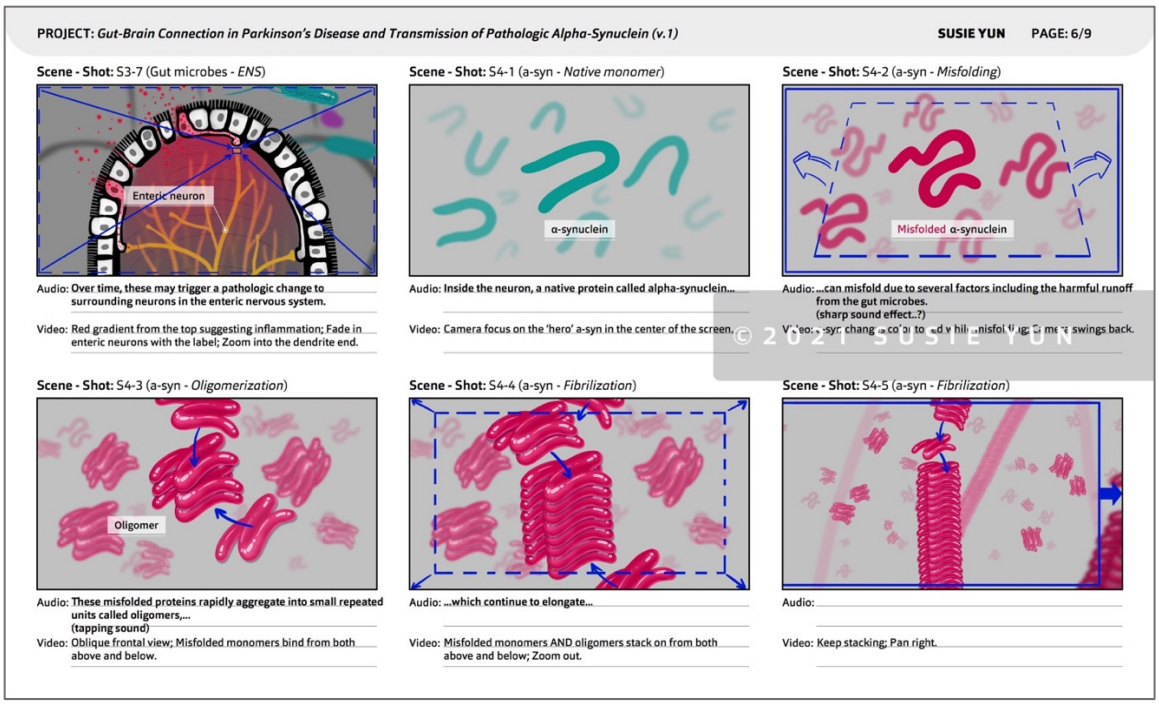


Figure 8. Selected storyboard for the 3D animation. This figure shows an example of the storyboards created for this project. Camera movements are indicated in blue. Text not intended to be read.

Following the storyboard development, a preliminary animation, also referred to as an Animatic, was created using the storyboard images and a rough recording of the voice over. The purpose of the animatic was to better visualize and determine final size relationships, camera movement, transitions and timing.

Asset Creation

i. Interactive Website

The interactive website including its 2D animations was created using Adobe Animate for interactivity and website accessibility. 2D assets were created using Adobe Illustrator as well as Adobe Animate for simple vector graphics. relevant stills

and graphic elements from the 3D Animation were added to the website after once available.

ii. 3D Animation

Prior to asset creation, production notes were developed for effective planning. 3D assets, including molecular models, were created using Cinema4D, Zbrush, PyMOL and ePMV. For molecular accuracy, 3D protein data were obtained from the Protein Data Bank (PDB). 3D visual assets were modeled in Zbrush and Cinema4D, composed in Cinema4D, and rendered with OctaneRender.

1. Creating a Small Intestine with Peristalsis

Following the introduction scene, the camera flies through the lumen of the small intestine, slowly approaching the lumen wall covered in villi. To create an accurate representation of the small intestine and provide a realistic perspective, endoscopic videos were referenced for peristaltic movement and texture. In Cinema 4D, a curved spline and a circle were drawn, and a **Sweep** generator was applied, creating a curved tube with equal diameter throughout. Then, a **Bulge** deformer (rotated 90°) was applied to the tube. To simulate natural constriction, the deformer's strength was adjusted to a negative value and fillet was checked (**Bulge > Object**). The bulge deformer was duplicated. An **Align to Spline** tag was applied to both deformers and linked to the spline previously used to create the

intestine allowing deformers to be animated along the axis of the tube (**Figure 9**).

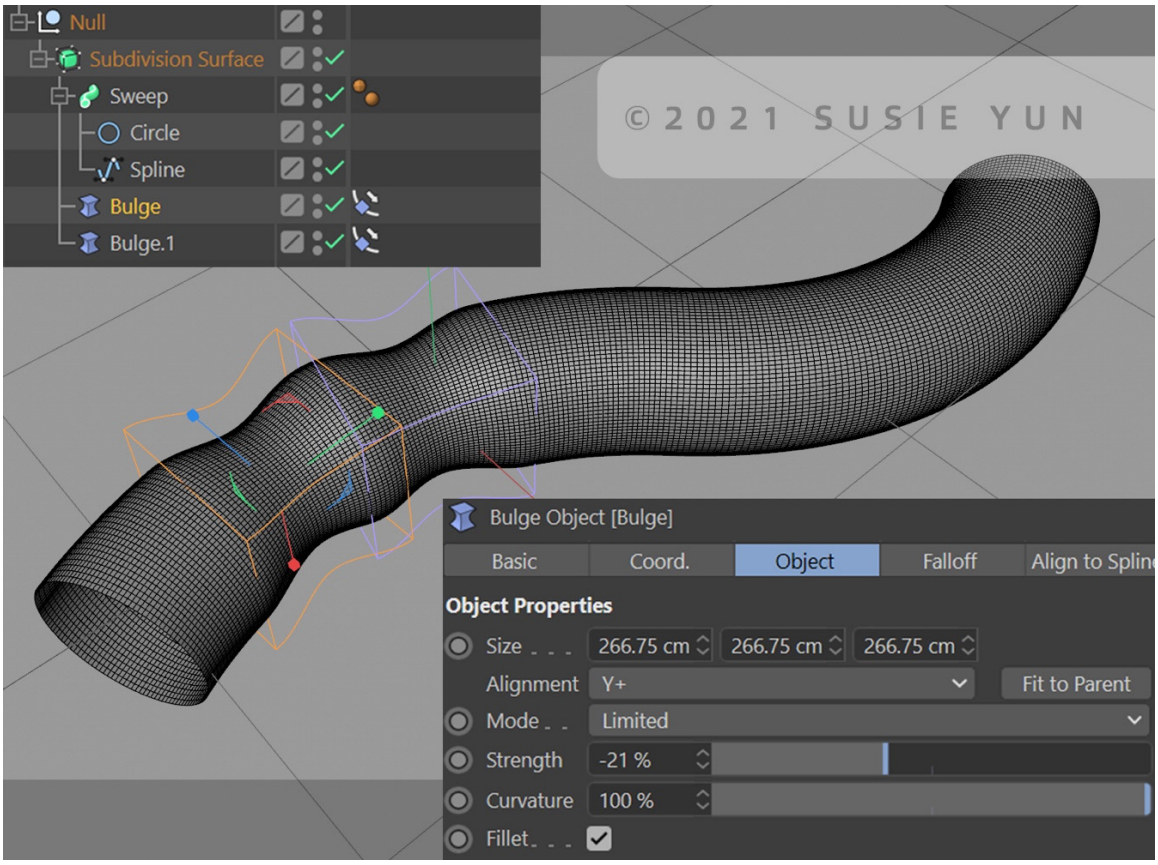


Figure 9. Creation of the small intestine with peristalsis. The object hierarchy and deformer attributes are shown.

Within the **Align to Spline** tag attributes, the 'position' value was keyframed so that one bulge deformer follows another with consistent distance (e.g. bulge in front: 17% → 95%, bulge following behind: 3% → 81%).

After animating the deformers, **subdivision surface** was applied to the tube to increase the polygon count. The model was made editable (**Connect object + delete**) and the circular folds were sculpted using a **Knife**

brush tool. The final sculpted model was then staged with lights and a camera (**Figure 10**).

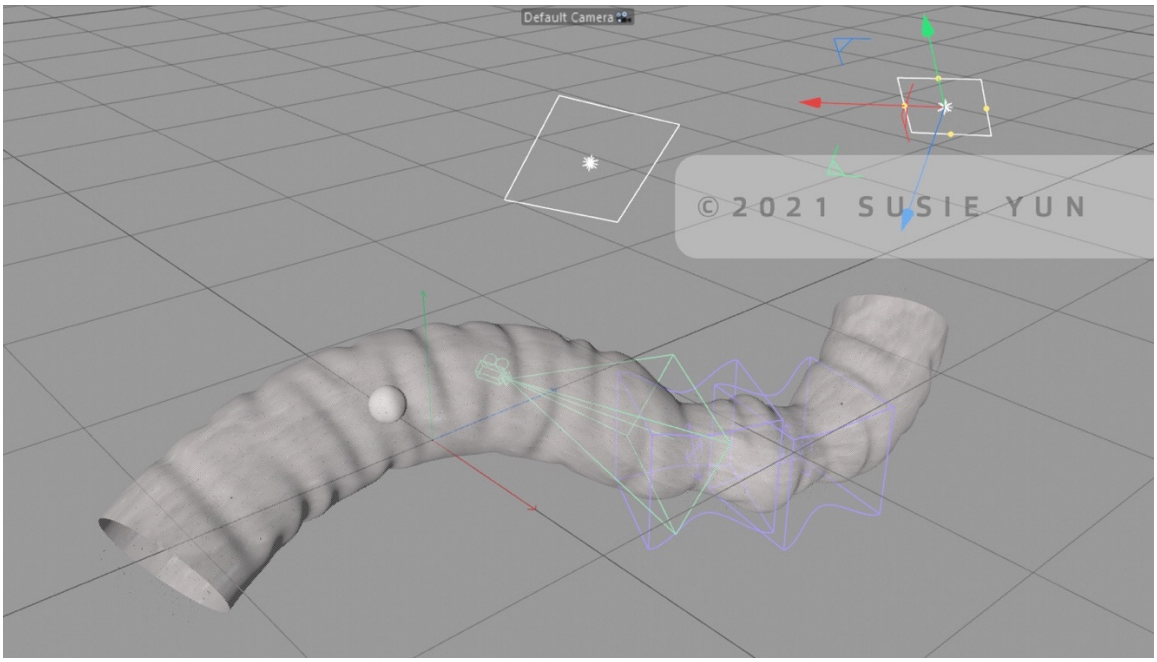


Figure 10. Final sculpted model of the small intestine. The lighting setup and camera position are also depicted.

Lastly, an **Octane Mix material** made of two glossy materials was created based on the endoscopic references and was applied to the finalized intestine model. **Material 1** and **Material 2** had different C4D native shaders (Naki and Voronoi 1 respectively) in different scales applied to their bump texture. The **Amount** node under the Octane Mix Material was connected to a gradient texture of noise (Octane) for a randomly scattered distribution of the two materials (**Figure 11** and **12**).

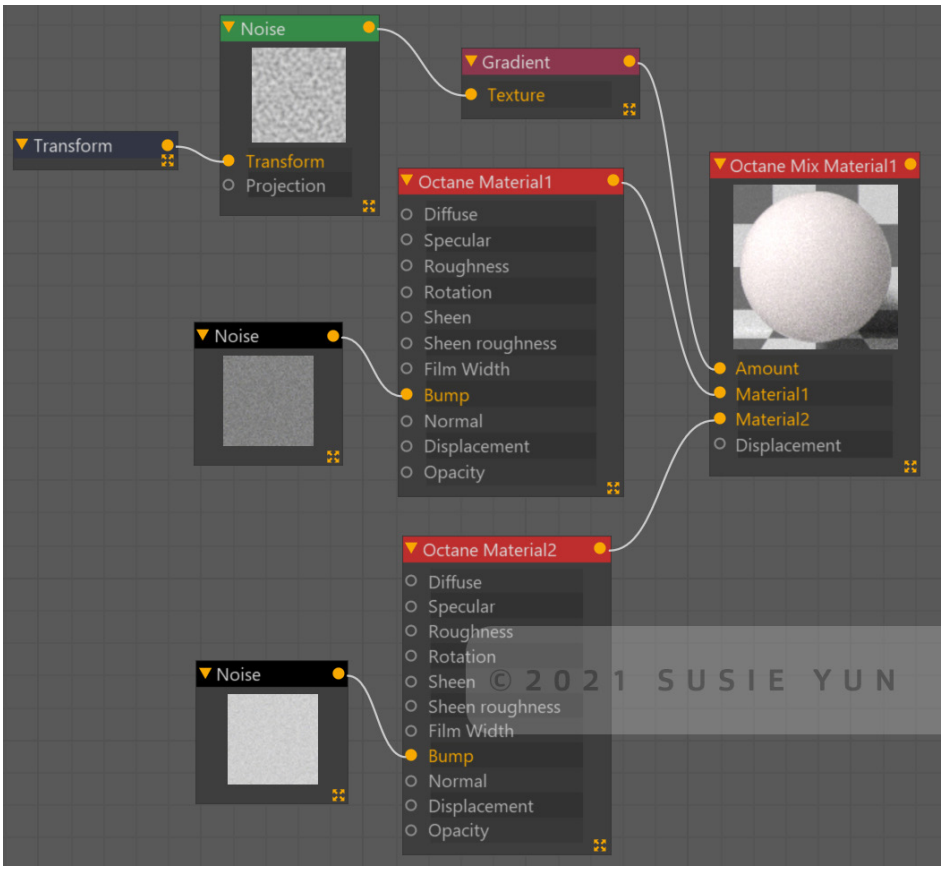


Figure 11. Node editor of the material applied to the small intestine model.

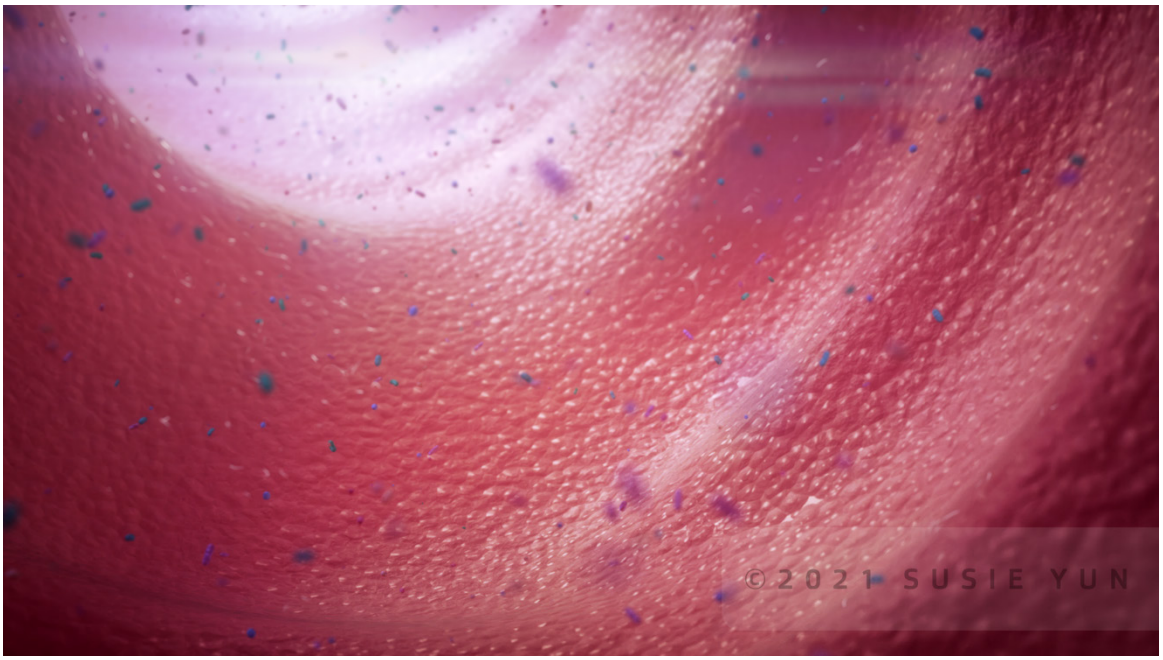


Figure 12. Octane render of the small intestine material. This image includes the cloned bacteria models which are not described in this paper.

2. Creating and Animating Villi

A scene with animated villi follows the previous scene where the camera zooms into the luminal wall. First, a plane was created in Cinema 4D and made editable. With all polygons selected, **Extrude Inner** ('Preserve group' unchecked) was used to extrude the polygons inwards to create a gap among the villi (**Figure 13**).

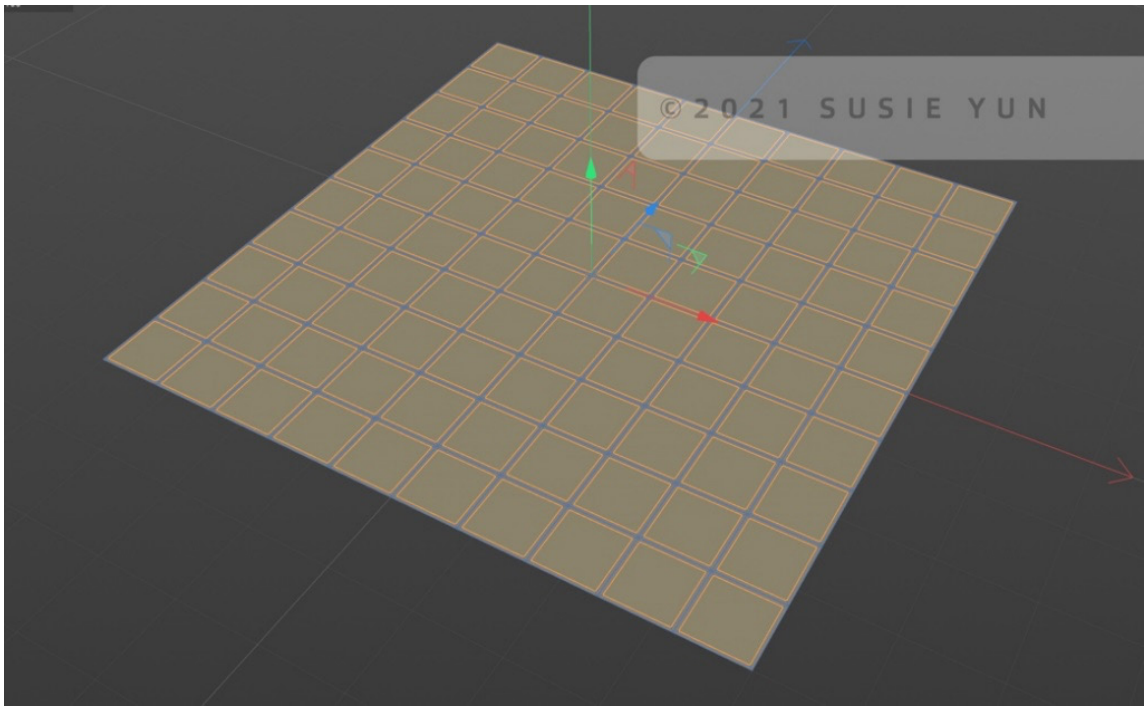


Figure 13. An editable plane with polygons extruded inwards.

With the extruded polygons still selected, a **Polygon Selection Tag** was added to the plane (**Select > Set selection**) to save the selection, which was then used to apply **MoGraph > MoExtrude** to the plane. The extrusion steps and transform attributes were adjusted to extrude the selected polygons in the positive *y*-axis, and the **subdivision surface** was applied (**Figure 14**).

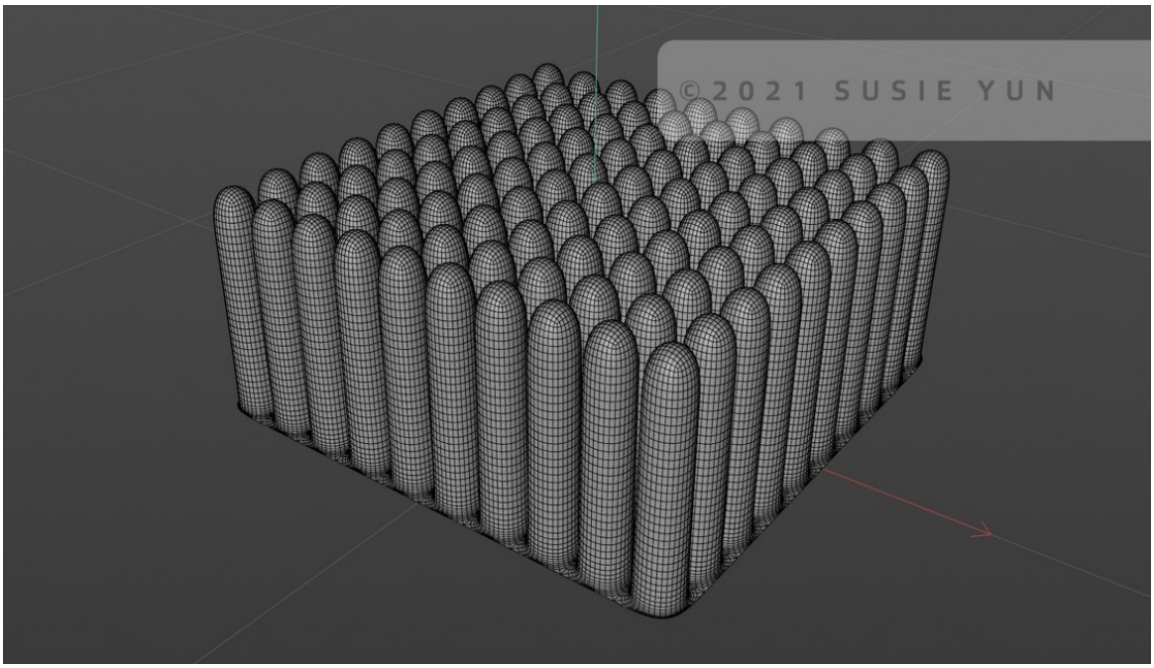


Figure 14. Extruded polygons with subdivision surface.

To create a larger colony of villi, **MoGraph > Cloner** was used in a grid array mode (iterate). To prevent overlap among the clones, the *x* and *z* value of the clone size was increased by the width of the plane (e.g. 400cm). Then, with the MoExtrude selected rather than the cloner, **MoGraph > Effector > Random** effector was applied to animate the villi movement as well as to randomly transform their rotation. The effector was set as Random with Noise mode in a Global space (Animation Speed: 18%) for a subtle movement of the villi, while the rotation parameters were increased in *H* and *P* values, resulting in **Figure 15**. Additionally, **MoGraph > Effector > Step** effector was applied and the spline graph was modified to vary the height of the villi.

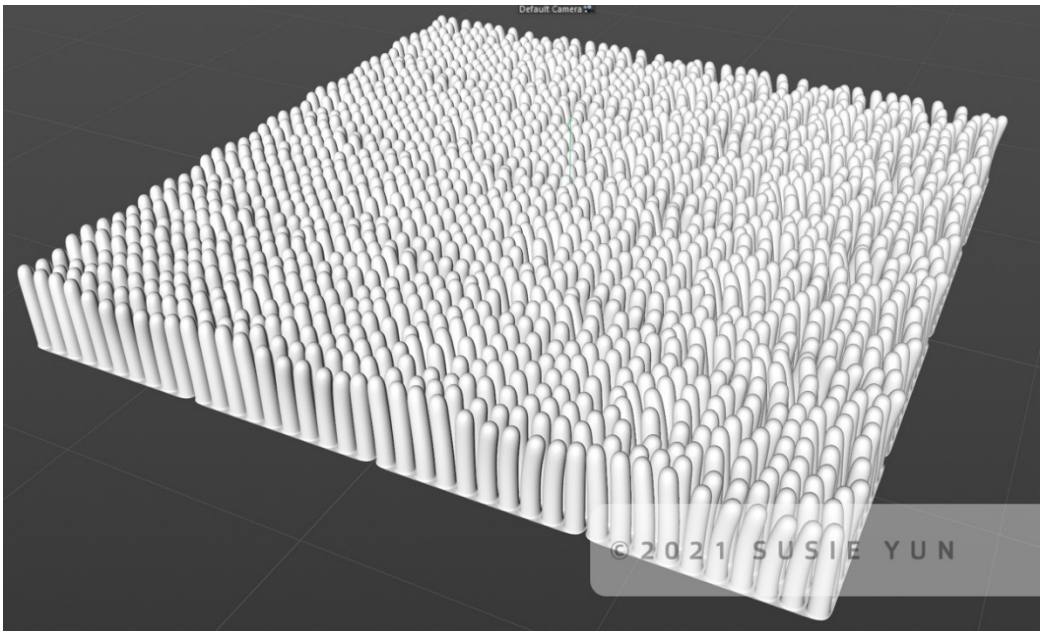


Figure 15. Cloned villi model with Random effector applied to MoGraph.

Finally, an **Octane Mix Material** made of two specular materials with subsurface scattering and a displacement texture was applied to the clones after introducing lights to the stage (**Figure 16**).

Figure 16. Octane render of the villi material. This image includes *E.coli* and the emitted particles which are not described in this paper.

3. Using PDB Data for Alpha-Synuclein and Misfolding

For molecular accuracy, PDB data was obtained for normal α-syn monomers (PDB ID: **1XQ8**) and misfolded α-syn oligomers (PDB ID: **2N0A**). Because there was no PDB data for a misfolded α-syn monomer, the oligomer PDB file was imported to PyMOL to isolate a monomer by **R-Clicking the chain > Chain > Copy to Object > New** (**Figure 17**), resulting in **Figure 18**.

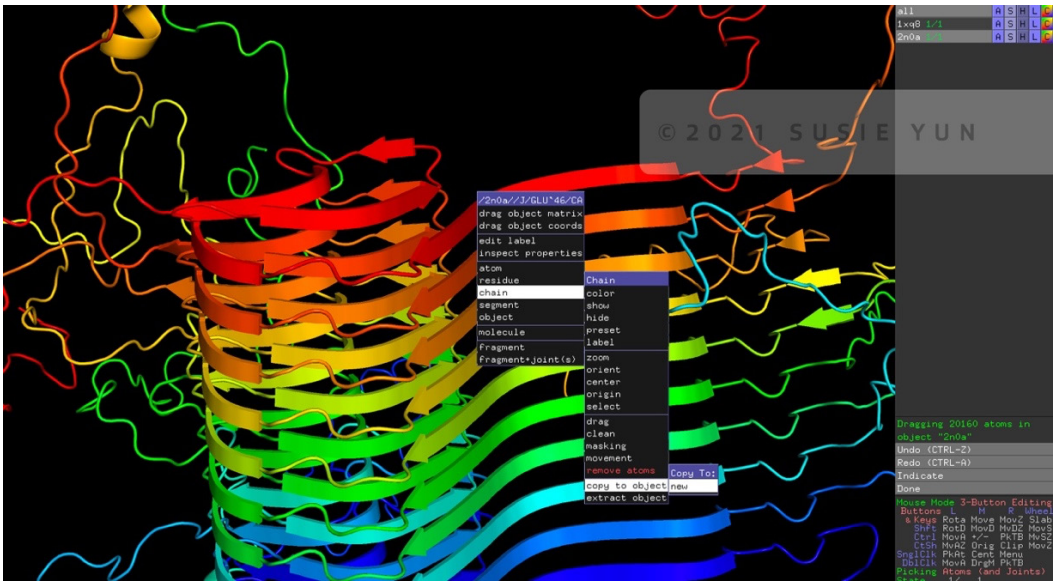


Figure 17. Isolation of a chain from the misfolded α-syn oligomer in PyMOL. Text not intended to be read.

Figure 18. Isolated misfolded α-syn monomer. Text not intended to be read.

The final PDB files were then exported and imported to C4D using ePMV. Instead of using the PDF files themselves as α-syn models, they were used as a "map" for tracing the shape of the proteins. For example, a spline was traced along the PDB molecule of the normal α-syn using the 4-grid viewport and then **Sweep** was applied using a small circle (**Figure 19**).

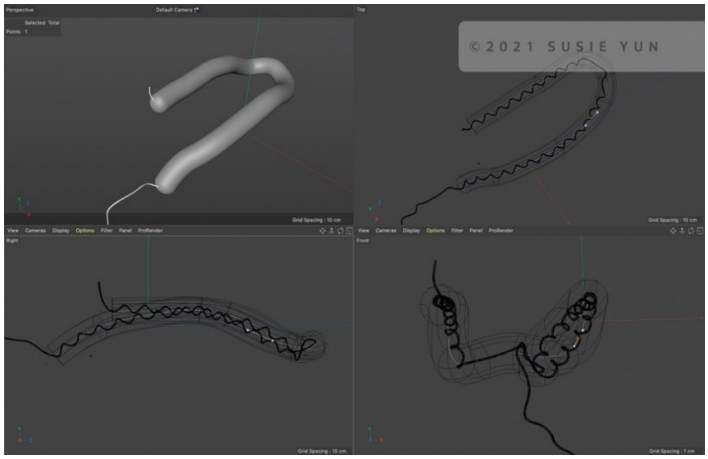


Figure 19. Sweep spline of the normal α-syn monomer.
Text not intended to be read.

By using **PoseMorph** (spline mode) and changing its blend value (100%), the points of the spline were modified using the PDB molecule of the misfolded α-syn monomer as a structural reference (**Figure 20**).

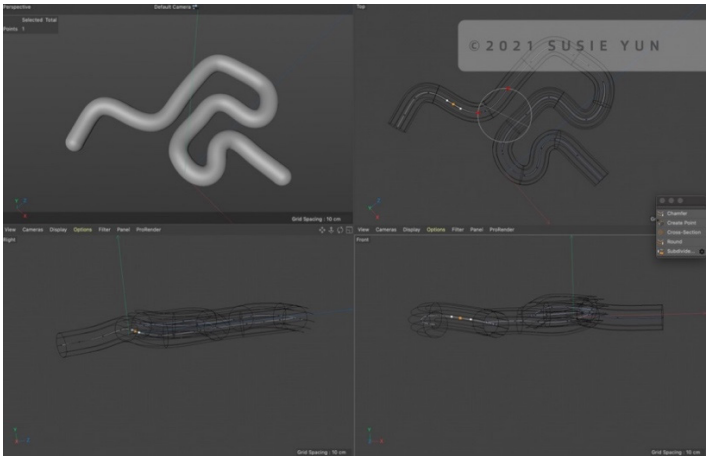


Figure 20. Sweep spline of the misfolded α-syn monomer.
Text not intended to be read.

Then, the **MSMSurf** surface representation was visualized using ePMV (**Figure 21**).

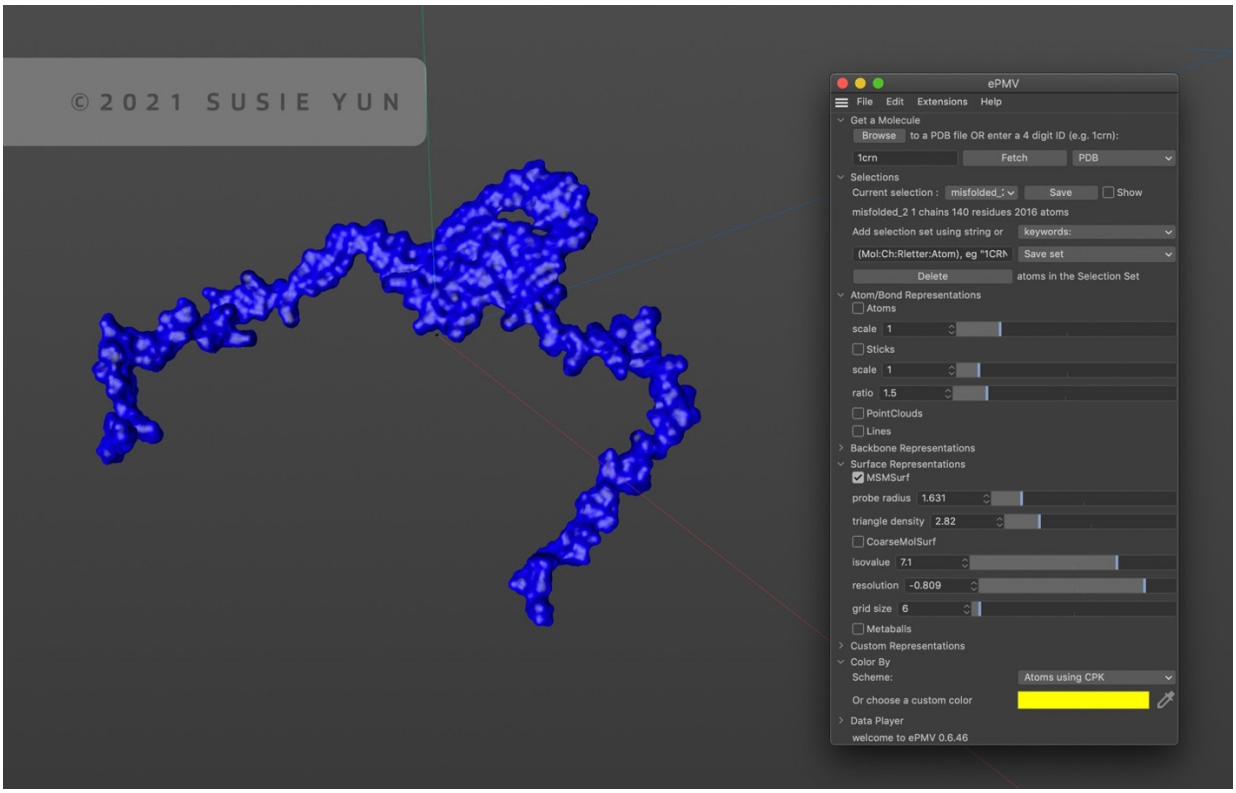


Figure 21. MSMSurf representation of the misfolded α-syn monomer. Text not intended to be read.

This was used as a reference for imitating the space-filling molecular representation of each α-syn monomers, by applying a **Displacer** deformer with noise and turbulence shader, resulting in a more simplified representation as shown in **Figure 22** and **23**. Once the models were finalized, PoseMorph was animated by keyframing the **blend** value (0% → 100%) to visualize the misfolding of the normal α-syn monomer.

Figure 22. Octane render of the displaced normal α-syn monomer.

Figure 23. Octane render of the misfolded α-syn monomer.

Post-production

i. Adobe After Effects

The post-production editing of the 3D animation was done in Adobe After Effects. This included compositing, color adjustments, text animations, and audio-to-video synchronization.

ii. Combination of Interactive Website and 3D Animation

The resulting 3D animation was imbedded into the *Research* page of the interactive website using Adobe Animate. As previously mentioned in *Asset Creation: Interactive Website*, selected stills and graphic elements were also added to the website (see *Results* section).

Interactive Website

Figure 24. Homepage of the interactive website.

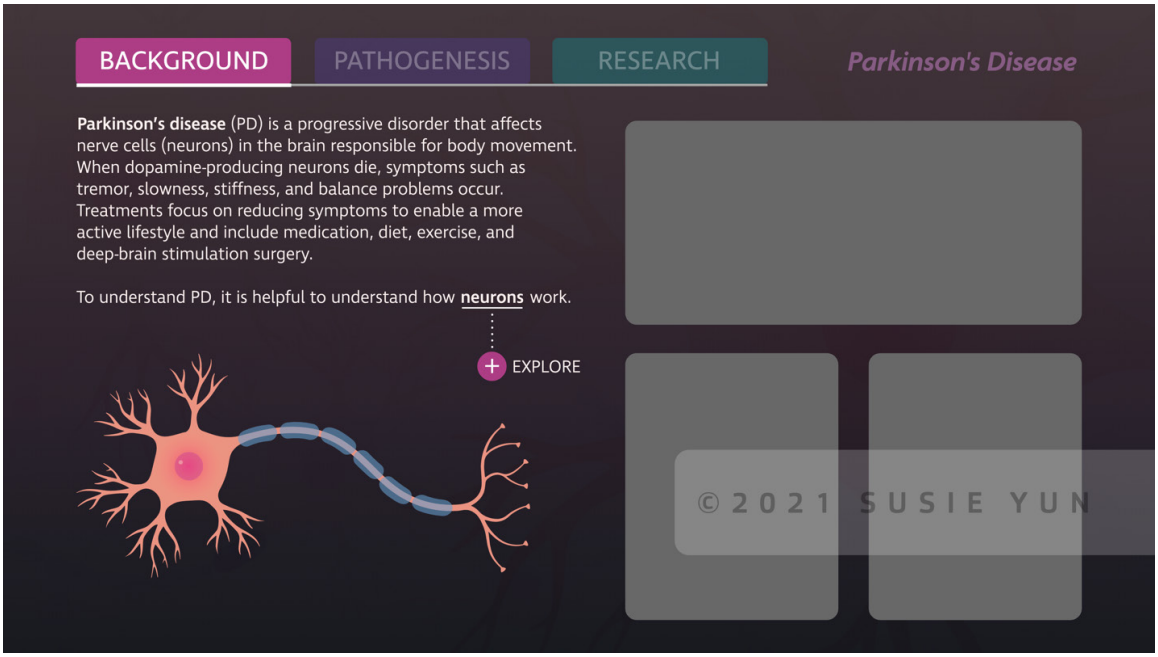


Figure 25. 'Background' page of the interactive website. Text not intended to be read.

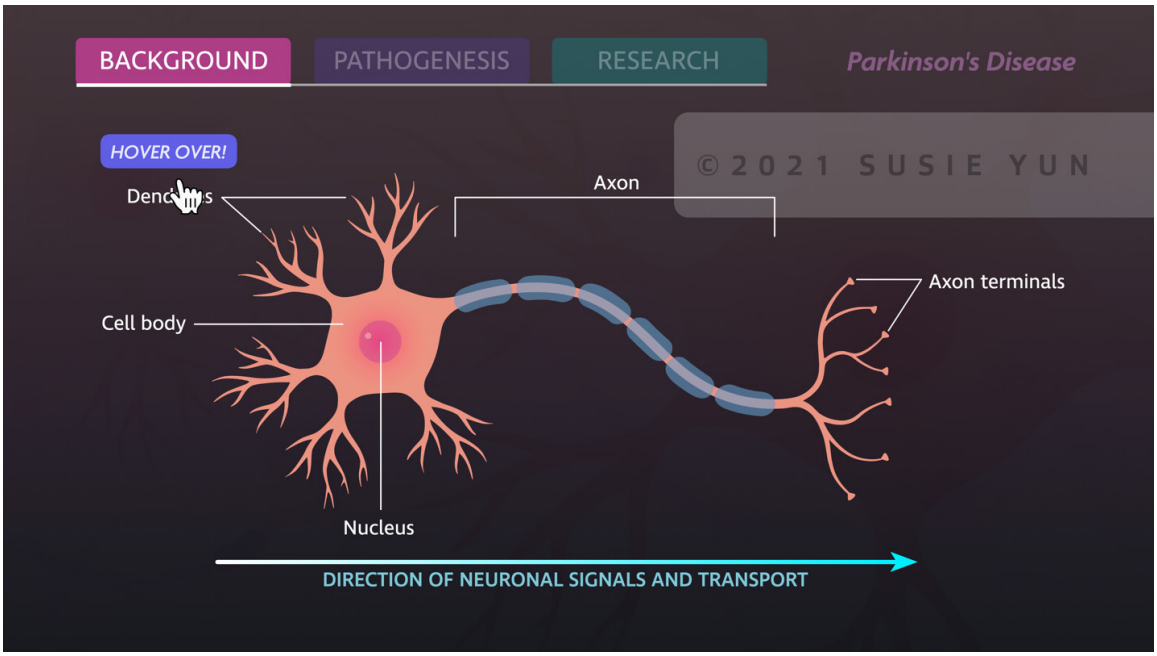


Figure 26. 'Explore' page of the interactive website with hover function. Text not intended to be read.

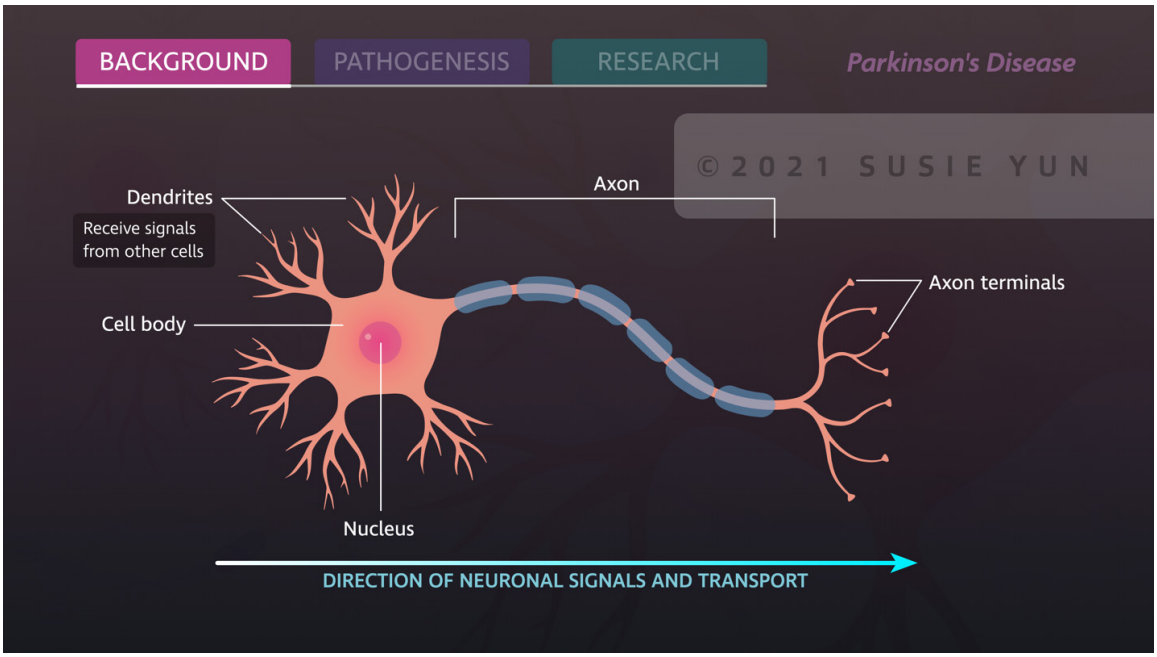


Figure 27. 'Explore' page of the interactive website with definitions. Text not intended to be read.

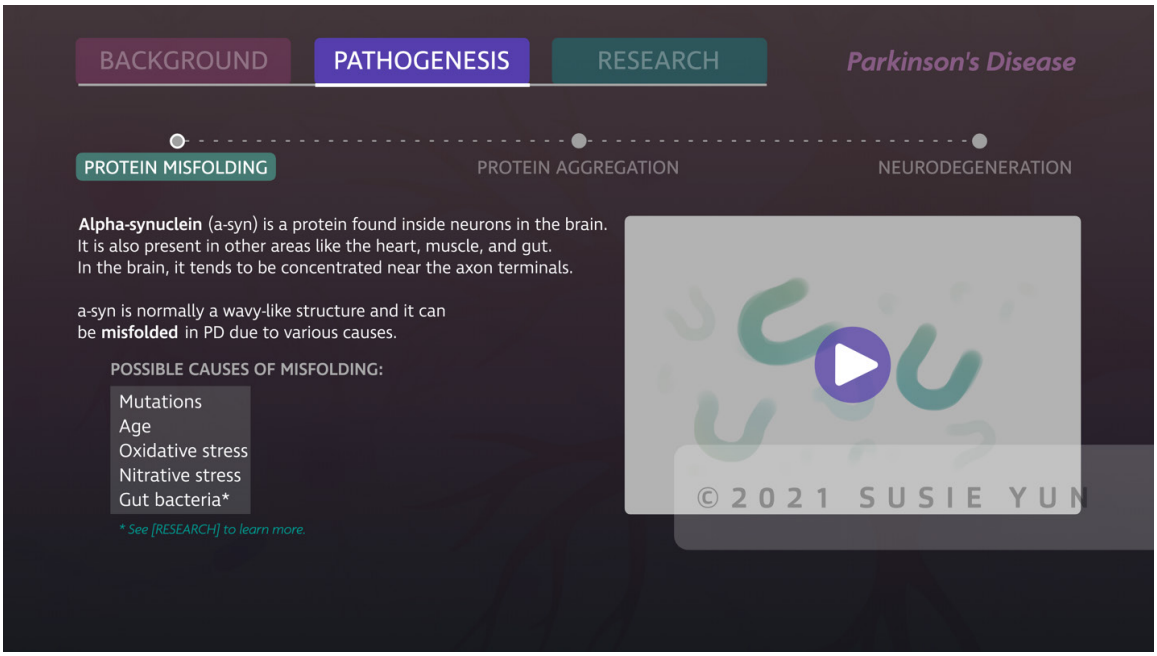


Figure 28. 'Pathogenesis' page of the interactive website. Text not intended to be read.

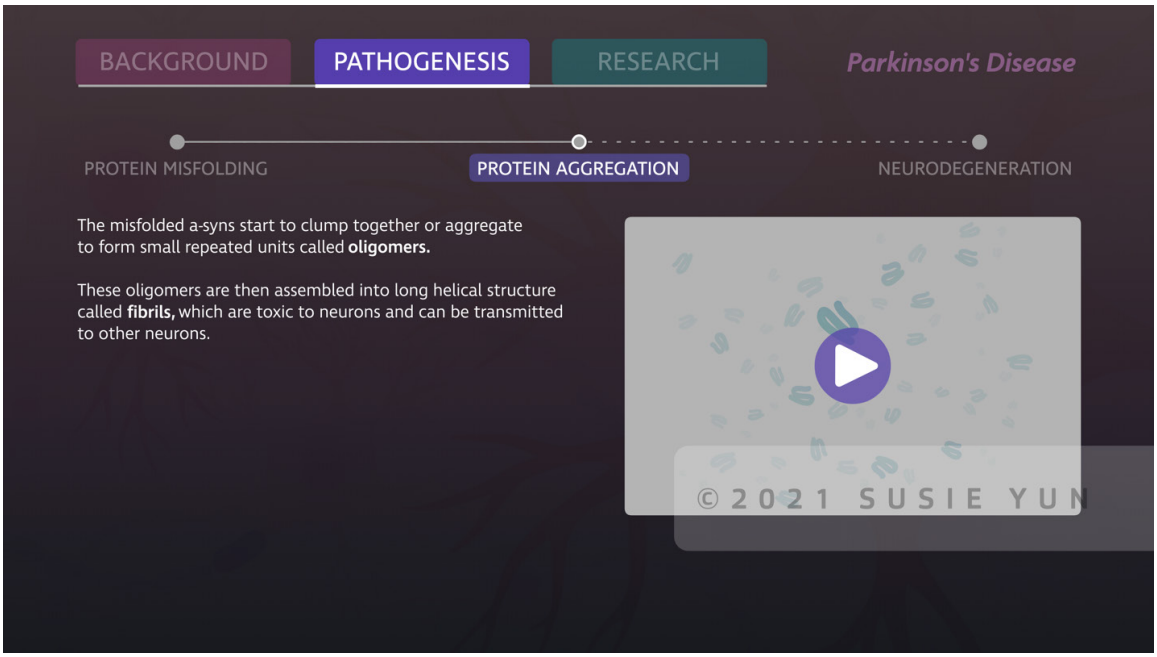


Figure 29. 'Protein Aggregation' page of the interactive website. Text not intended to be read.

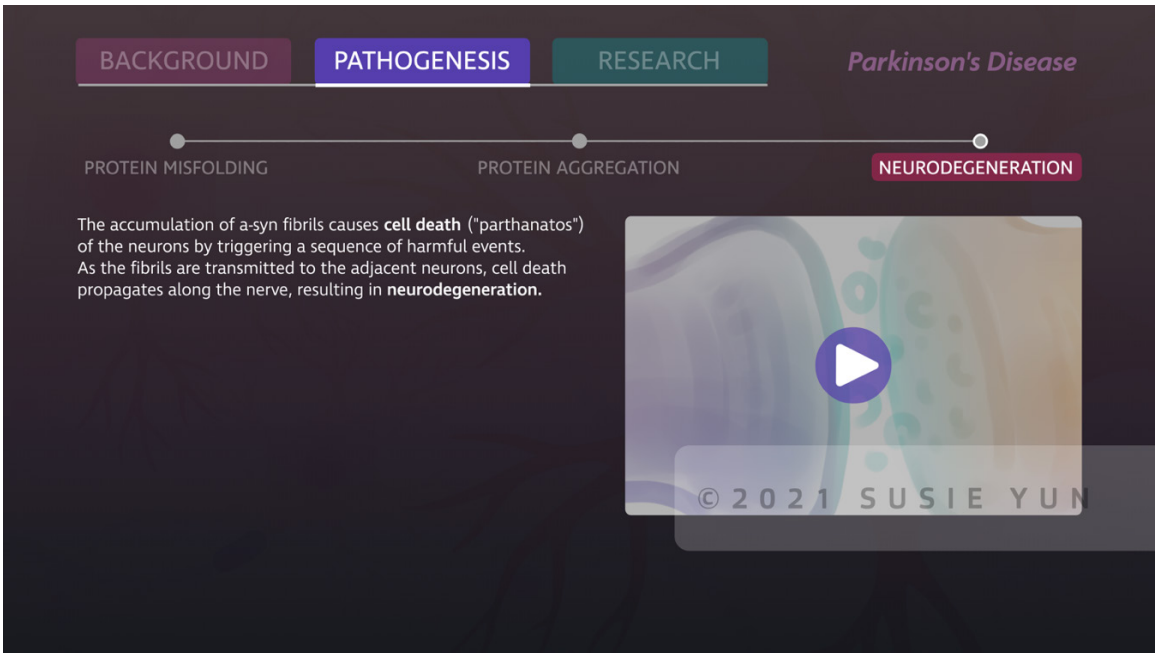


Figure 30. 'Neurodegeneration' page of the interactive website. Text not intended to be read.

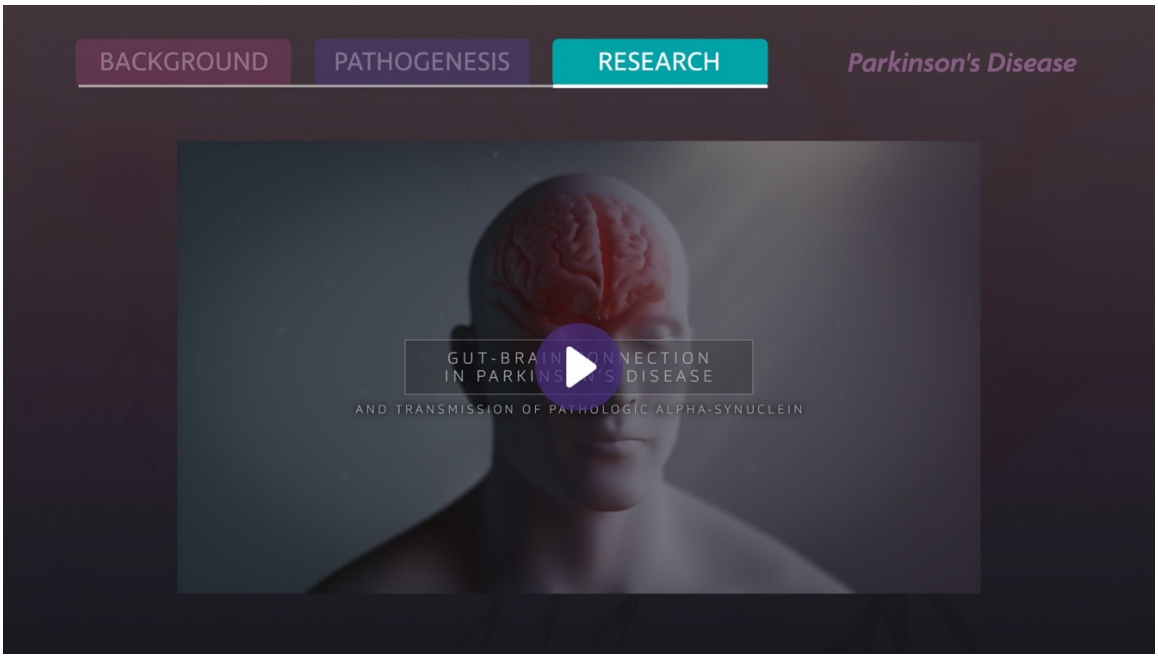


Figure 31. 'Research' page of the interactive website. Text not intended to be read.

3D Animation Stills

Figure 32. 3D Animation still. Title scene. Text not intended to be read.

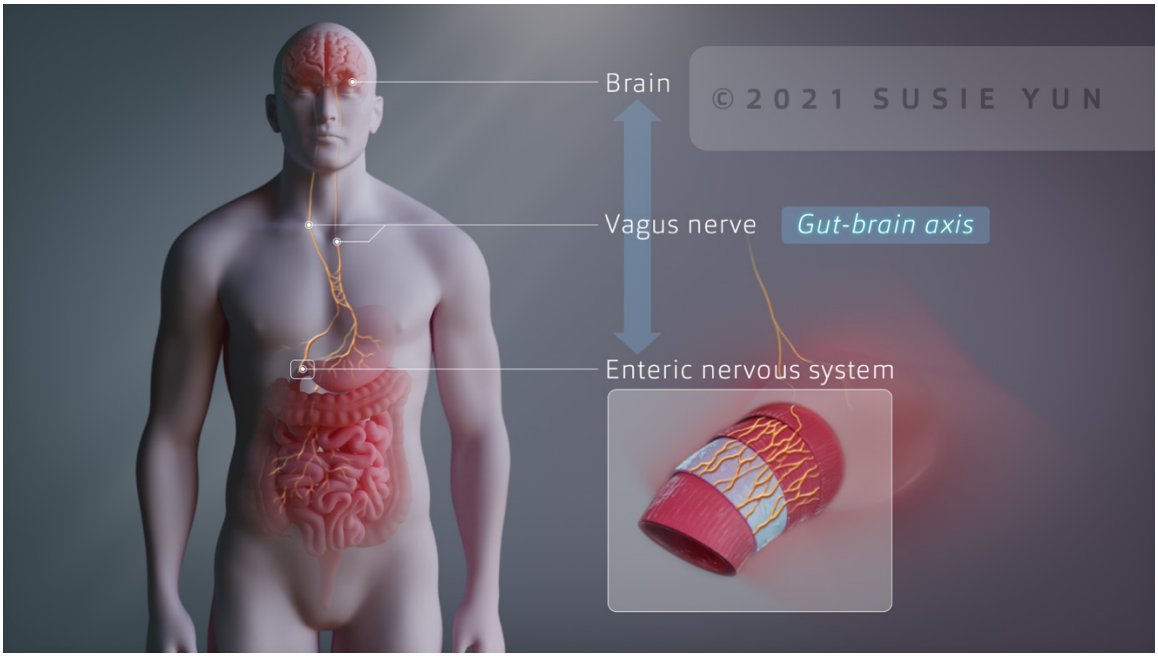


Figure 33. 3D Animation still. Gut-brain axis is defined.

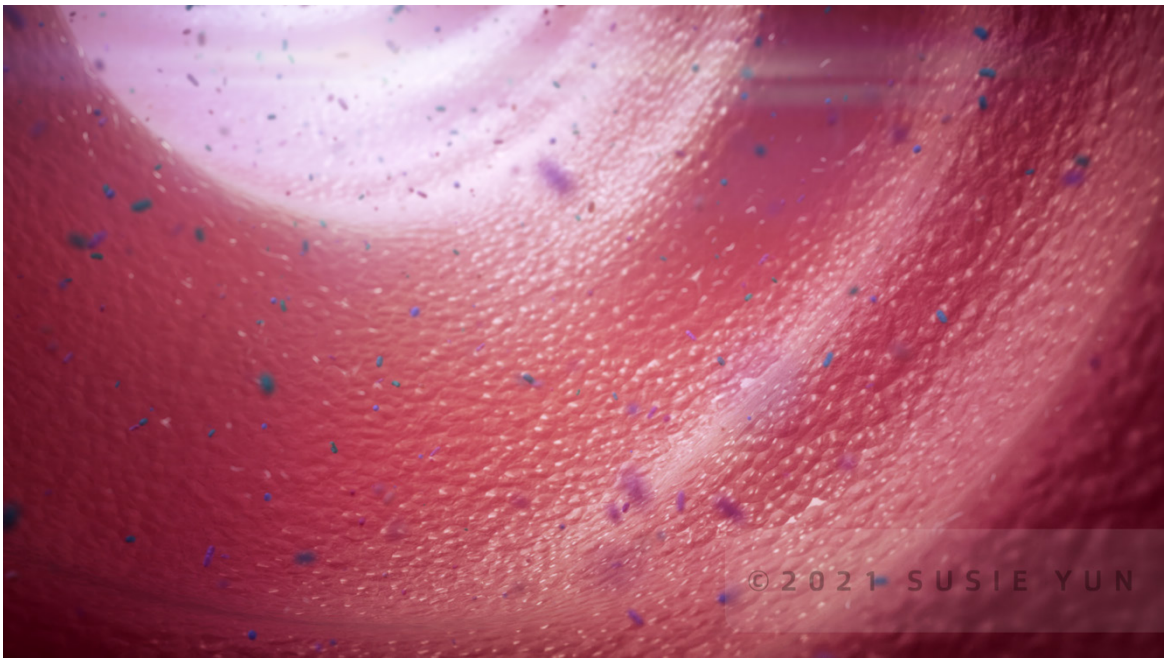


Figure 34. 3D Animation still. Luminal wall of the small intestine is brought into focus.

Figure 35. 3D Animation still. Intestinal villi with *E.coli* releasing harmful substances is shown.

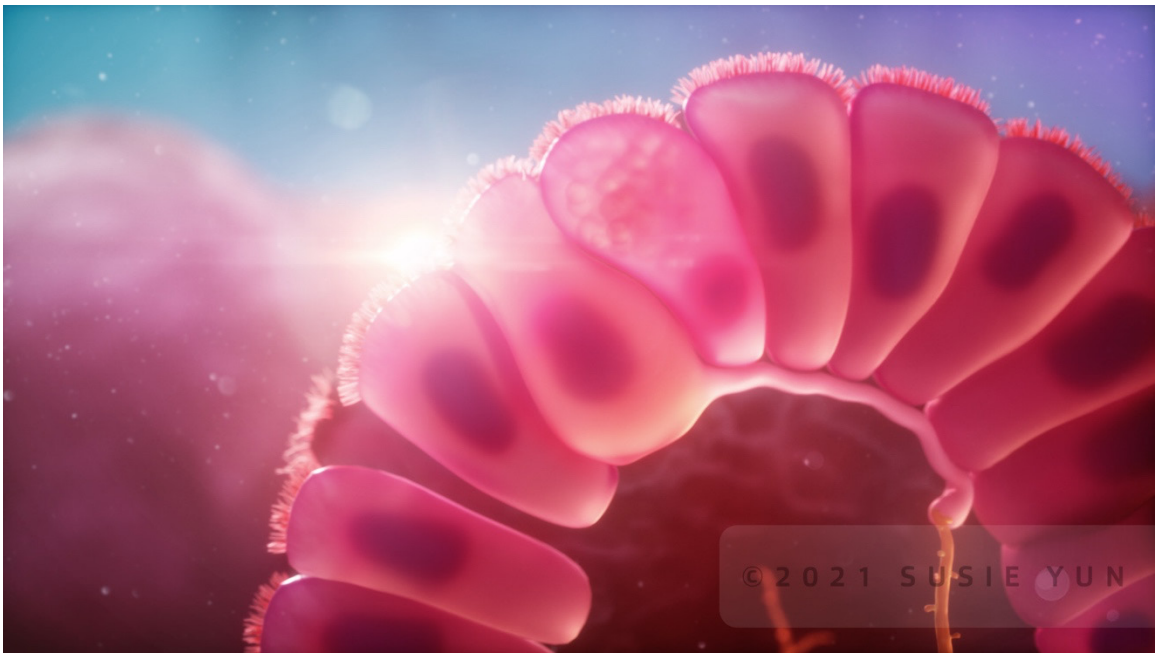


Figure 36. 3D Animation still. Cross-section of a villus is shown.

Figure 37. 3D Animation still. A network of enteric neurons is shown.

Figure 38. 3D Animation still. A normal α-syn monomer is brought into focus.

Figure 39. 3D Animation still. Misfolded α-syn monomers are shown.

Figure 40. 3D Animation still. Misfolded α-syn oligomers are shown.

Figure 41. 3D Animation still. Formation of α-syn fibrils is shown.

Access to Assets Resulting from this Thesis

The final 3D animation resulting from this thesis can be viewed at https://www.thedawsonlab.org/ and https://susieyun.com/. The author of this project can be reached through the Department of Art as Applied to Medicine via the website https://medicalart.johnshopkins.edu/.

Discussion

Project Objectives

The primary purpose of this project was to develop a 2D interactive educational platform for the scientific background and pathogenesis of Parkinson's Disease (PD), and to create a narrative 3D animation highlighting the recent research regarding PD, and the potential gut-brain connection. While the content was based on a vast amount of research, the narration was simplified to a level of complexity appropriate for PD patients and the lay public to effectively communicate this important topic. The final 3D animation describing the gut-brain connection resulted in a video file of 4 minutes and 5 seconds in duration (without credits). The use of multimedia was essential in this project as the introductory teaching module in the interactive website (i) provided necessary information needed prior to understanding a complex research topic; and (ii) helped shorten the length of the 3D animation which compensates for the short attention span of PD patients.

Challenges During the Project

There were two main challenges during the script development: (i) the topic of gut-brain connection in PD is very complex and potentially difficult to understand for the target audience, as it involves neuroscience, molecular biology, and pathobiology; (ii) there are many "unknowns" in the in the current body of research which added to the difficulty in explaining the topic. To overcome these challenges,

the script was developed using plain language and explanations utilizing broad terminology rather than highly specific, detailed hypotheses and mechanisms.

There were further challenges in creating 3D assets. The physical and morphological anatomy of the ENS was challenging to visualize in 3D, as the existing visual resources are simplistic, inaccurate, and limited. These challenges were addressed by reaching out to external content experts and acquiring advanced microscopy and fluoroscopy references.

Future Directions

Future directions for this project include completing the interactive website with fully functional interactivity not addressed in this project. Among the three 2D animations in the 'Pathogenesis' section of the website, only one has been animated for the scope of this project. In addition, the website was published as a local directory rather than for public accessibility. Making the interactive website public will broaden the audience and enhance the functionality of the project.

Furthermore, making the interactive website compatible in a mobile format may improve accessibility. The use of multimedia in this project can be implemented in other topics of patient education as well.

Appendix A: 3D Animation Script

Parkinson's disease, or PD, is the second most common neurodegenerative disorder, affecting the brain and other parts of the body. It is commonly seen as a movement disorder due to its prominent motor symptoms, such as slow movement, muscle rigidity, and rest tremor.

However, there are also many non-motor symptoms often observed in Parkinson's patients, such as loss of smell, digestive problems, and sleep disorders. Some of the most common are digestive problems, including constipation, bloating, and abdominal pain. These problems may be present years before the onset of motor symptoms. In other words, they may indicate the early stages of Parkinson's and provide an early warning system for patients and healthcare providers.

While Parkinson's disease is considered a brain problem, mounting evidence suggests it may actually begin in the digestive system. Therefore, it is more important than ever to uncover and understand the relationship between the gut and the brain.

The enteric nervous system, which controls the function of the gut, communicates with the brain via the vagus nerve. This nerve forms the gut-brain axis, a two-way highway where messages are transmitted in both directions. The gut-end of this nerve appears to be the origin of a pathogenic protein that can propagate to the brain, possibly causing a variety of neurodegenerative diseases including Parkinson's disease. While it is yet unclear what triggers this pathogenic protein to develop, several factors have been identified. Here is one possible scenario:

The gut is colonized with a massive community of microbes. Some of which may produce harmful substances that can enter the cells lining the gut or pass through the leaky barrier. Over time, these may trigger a pathologic change to surrounding neurons in the enteric nervous system.

Inside the neuron, a native protein called alpha-synuclein can misfold due to several factors including the harmful runoff from the gut microbes. These misfolded proteins rapidly aggregate into small, repeated units called oligomers, which continue to elongate and assemble into long fibrils. Once formed, the fibrils can spread and be transmitted from neuron to neuron.

Once inside an adjacent neuron, the fibrils act as templates to seed misfolding of normal alpha-synuclein proteins, causing the entire process to repeat. The fibrils are toxic to neurons and eventually cause parthanatos, a type of cell death.

After repeated steps of misfolding, aggregation, and transmission, more and more neurons along the vagus nerve accumulate the toxic fibrils and die. With time, the pathology propagates to reach the brain ultimately causing the symptoms associated with Parkinson's disease.

Although the cause of the initial misfolding in the gut remains unclear, the gut-to-brain spread of the pathogenic protein as well as the early onset of digestive problems suggest that Parkinson's may start in the gut. Further research may lead to early diagnosis of Parkinson's disease and pave the way for effective early interventions that target the gut instead of the brain.

Appendix B: 3D Animation Storyboards

The 3D animation storyboards were created according to the following order:

1. Introduction
2. Gut-brain connection
3. Gut microbes
4. Alpha-synuclein
5. Fibril toxicity and cell death (Parthanatos)
6. Neurodegeneration
7. Conclusion

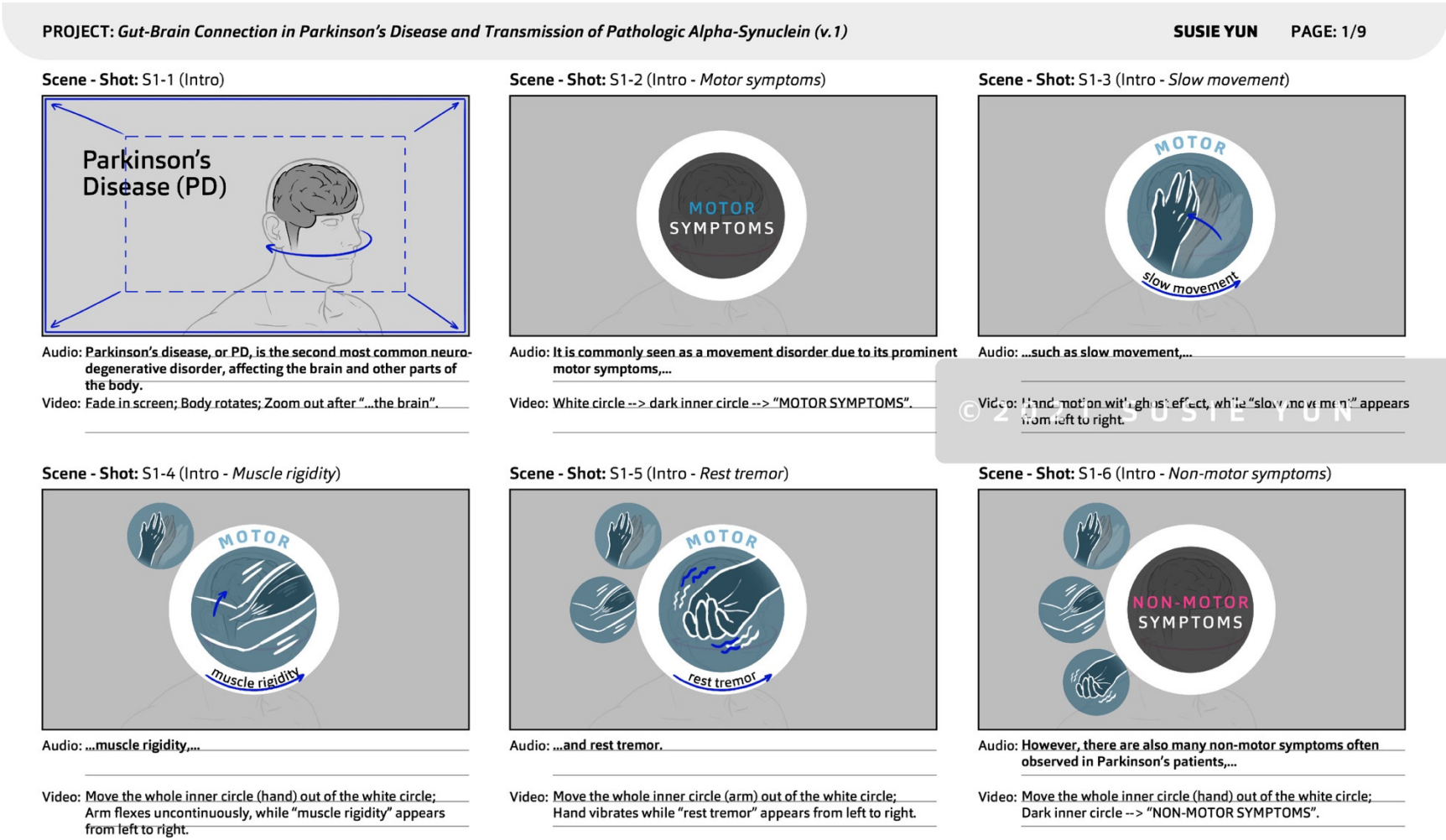


Figure 42. 3D Animation storyboard, page 1. Text not intended to be read.

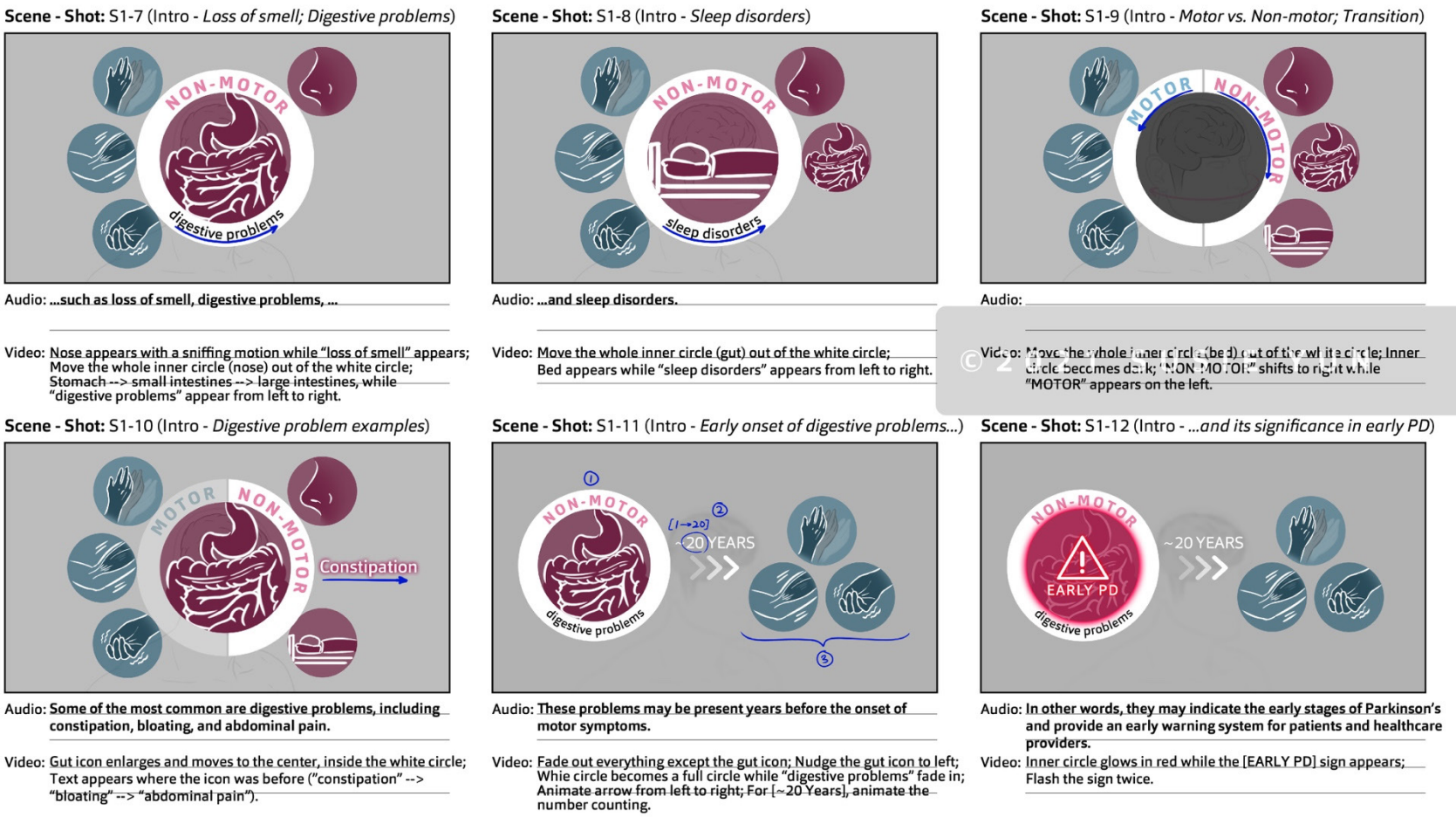


Figure 43. 3D Animation storyboard, page 2. Text not intended to be read.

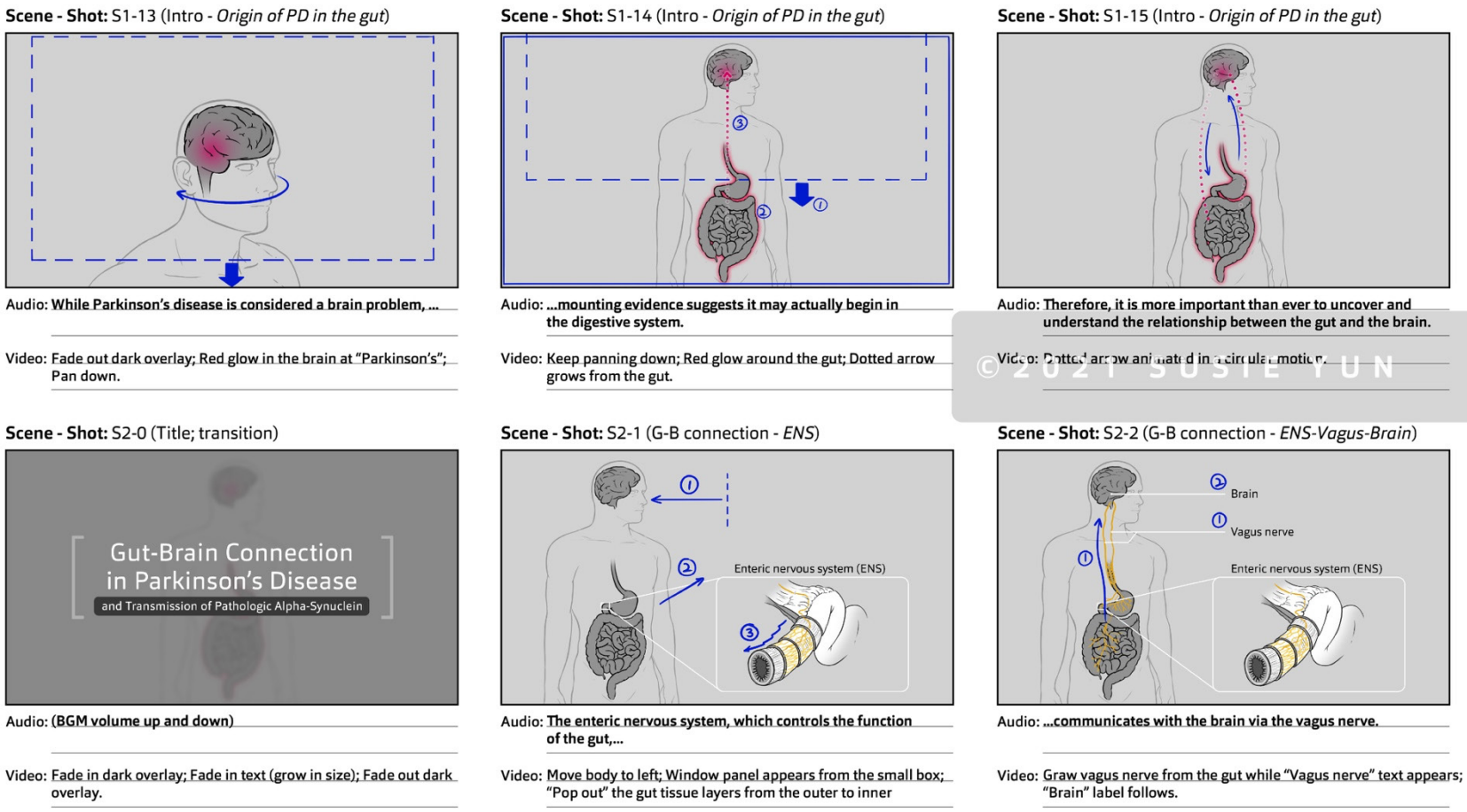


Figure 44. 3D Animation storyboard, page 3. Text not intended to be read.

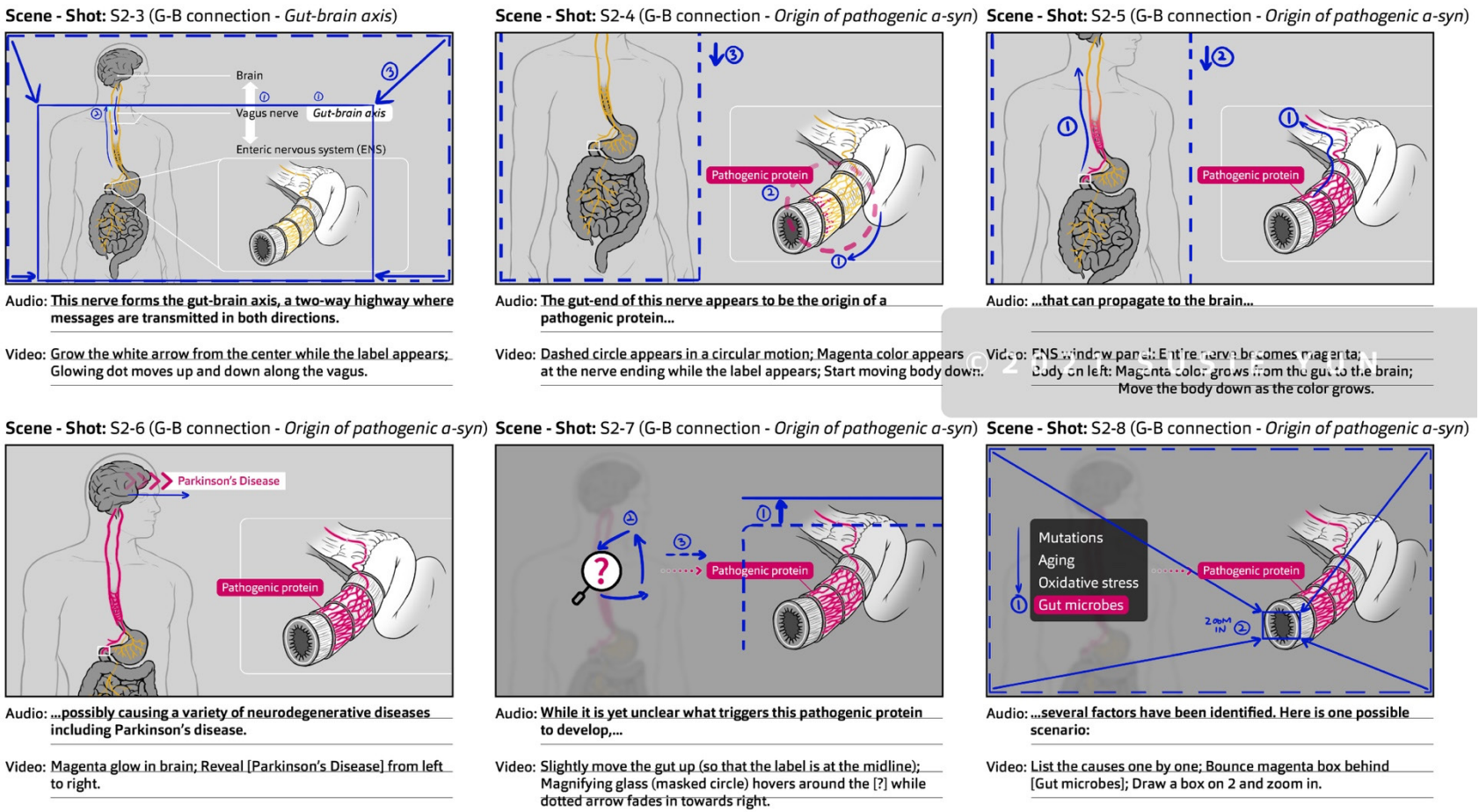
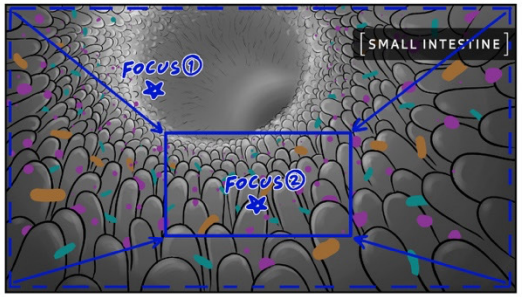


Figure 45. 3D Animation storyboard, page 4. Text not intended to be read.

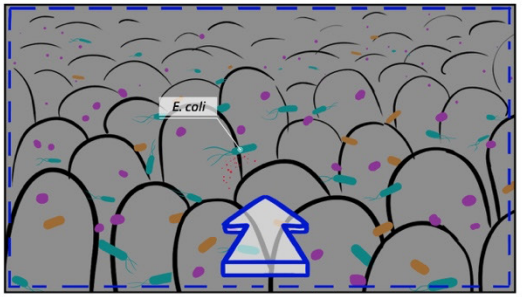
Scene - Shot: S3-1 (Gut microbes - *Establishing shot*)



Audio: **The gut is colonized with a massive community of microbes. (underwater sound)**

Video: Camera moves forward while keeping focus at 1; Shift focus to 2 at "community".

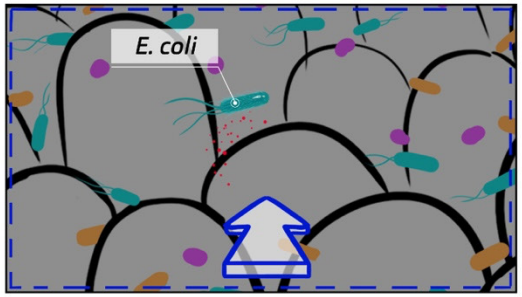
Scene - Shot: S3-2 (Gut microbes - *Action shot*)



Audio: **Within this colony,...**

Video: Camera moves forward; Camera focus on the 'hero' E.coli; Red particles falling from the 'hero'.

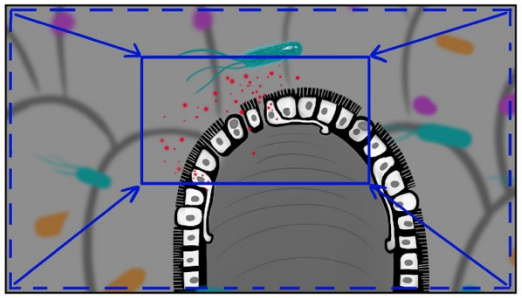
Scene - Shot: S3-3 (Gut microbes - *Action shot*)



Audio: **...some bacteria may produce...**

Video: Camera moves forward

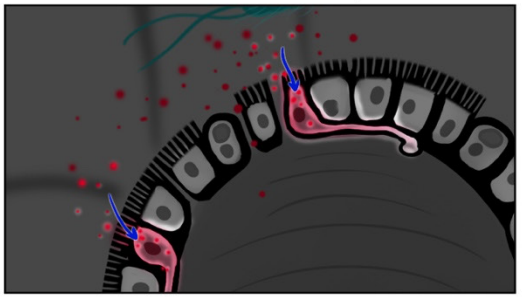
Scene - Shot: S3-4 (Gut microbes - *Tight shot*)



Audio: **...harmful substances that can...**

Video: Show cross-section of the villus with the epithelium highlighted; Zoom in to the leaky junction.

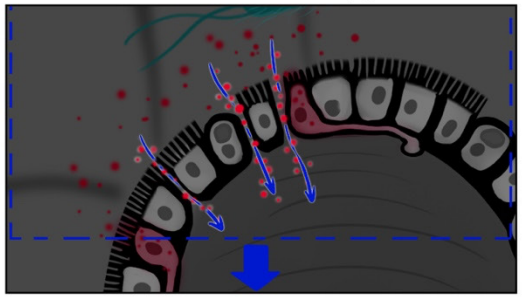
Scene - Shot: S3-5 (Gut microbes - *Neuropod cells*)



Audio: **...enter the cells lining the gut...**

Video: Darken everything except the neuropod cells and the particles entering them.

Scene - Shot: S3-6 (Gut microbes - *Leaky gut*)

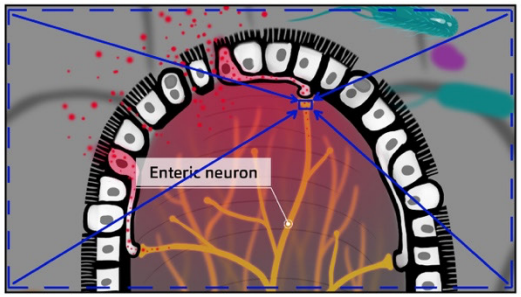


Audio: **...or pass through the leaky barrier.**

Video: Darken everything except the particles moving through the gap; Pan down.

Figure 46. 3D Animation storyboard, page 5. Text not intended to be read.

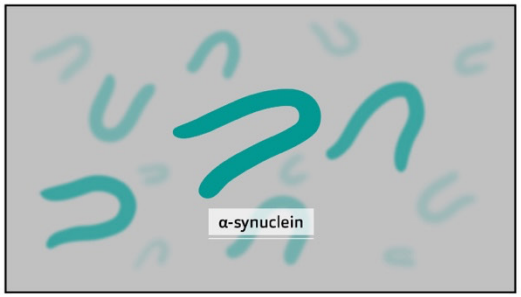
Scene - Shot: S3-7 (Gut microbes - *ENS*)



Audio: **Over time, these may trigger a pathologic change to surrounding neurons in the enteric nervous system.**

Video: Red gradient from the top suggesting inflammation; Fade in enteric neurons with the label; Zoom into the dendrite end.

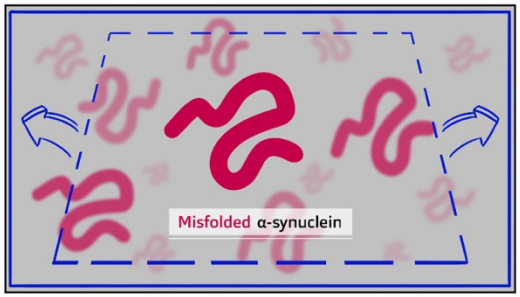
Scene - Shot: S4-1 (a-syn - *Native monomer*)



Audio: **Inside the neuron, a native protein called alpha-synuclein...**

Video: Camera focus on the 'hero' a-syn in the center of the screen.

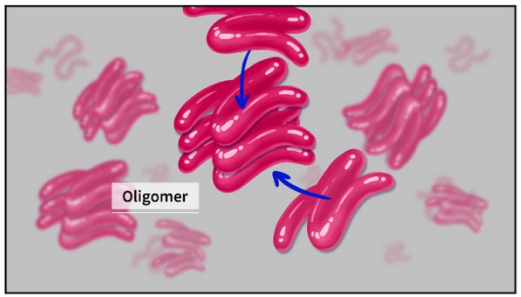
Scene - Shot: S4-2 (a-syn - *Misfolding*)



Audio: **...can misfold due to several factors including the harmful runoff from the gut microbes.**
(sharp sound effect..?)

Video: a-syn changes color to red while misfolding. Camera swings back.

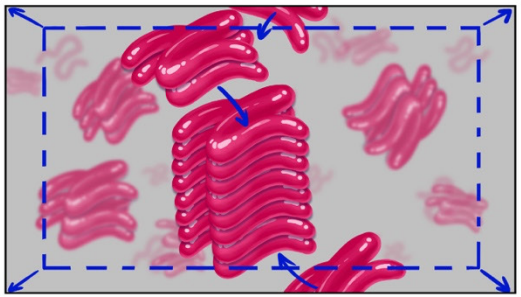
Scene - Shot: S4-3 (a-syn - *Oligomerization*)



Audio: **These misfolded proteins rapidly aggregate into small repeated units called oligomers,...**
(tapping sound)

Video: Oblique frontal view; Misfolded monomers bind from both above and below.

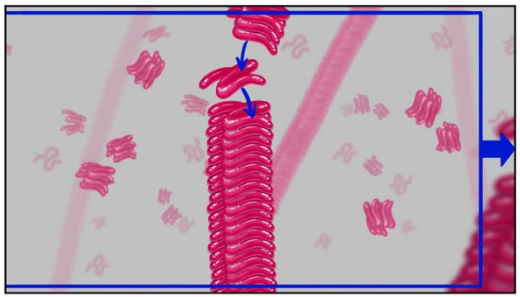
Scene - Shot: S4-4 (a-syn - *Fibrilization*)



Audio: **...which continue to elongate...**

Video: Misfolded monomers AND oligomers stack on from both above and below; Zoom out.

Scene - Shot: S4-5 (a-syn - *Fibrilization*)

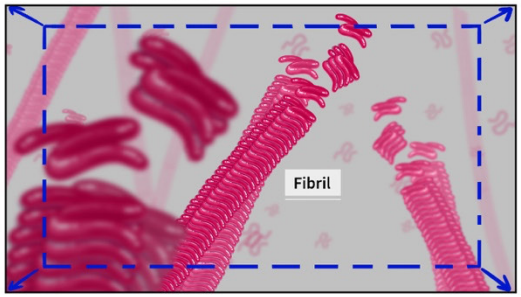


Audio:

Video: Keep stacking; Pan right.

Figure 47. 3D Animation storyboard, page 6. Text not intended to be read.

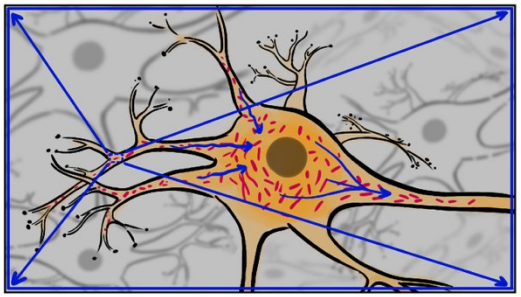
Scene - Shot: S4-6 (a-syn - *Fibrilization*)



Audio: ...and assemble into long fibrils.

Video: Dimerized protofibrils; Misfolded monomers and oligomers keep binding; Zoom out.

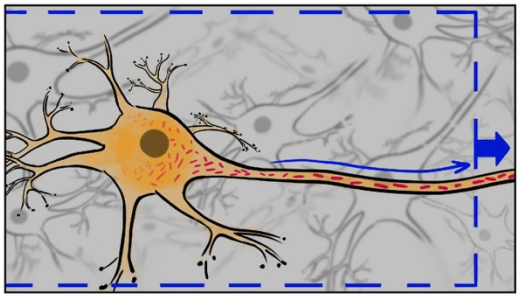
Scene - Shot: S4-7 (a-syn - *Transmission*)



Audio: Once formed, the fibrils can spread...

Video: Zoom out to the full view of the neuron;
Focus on the 'hero' neuron with the fibrils moving towards cell body;
*Some remain in the cell body, some move towards axon.

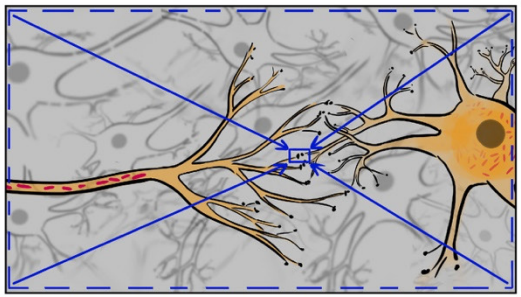
Scene - Shot: S4-8 (a-syn - *Transmission*)



Audio:

Video: Fibrils moving towards the axon terminal; Camera follows (pan right).

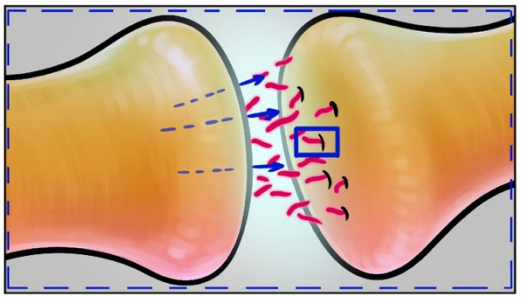
Scene - Shot: S4-9 (a-syn- *Transmission*)



Audio: ...and be transmitted...

Video: Zoom into the synapse.

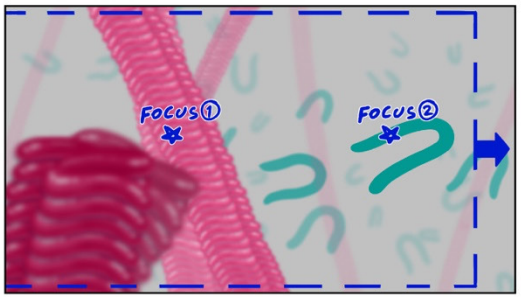
Scene - Shot: S4-10 (a-syn- *Transmission*)



Audio: ...from neuron to neuron.

Video: Fibrils enter the post-synaptic neurons via receptor-mediated endocytosis; Show a bind & pull action; Zoom in.

Scene - Shot: S5-1 (Toxicity & Cell Death - *Seeding*)



Audio: Once inside an adjacent neuron, the fibrils act as a template...

Video: Shift focus from 1 to 2 after "template"; Pan right.

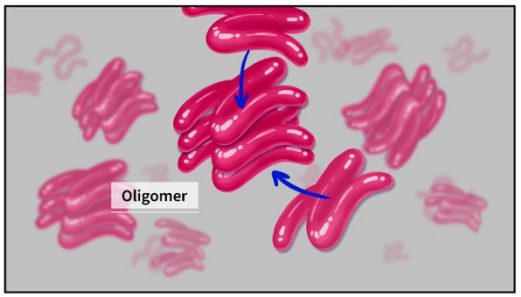
Figure 48. 3D Animation storyboard, page 7. Text not intended to be read.

Scene - Shot: S5-2 (Toxicity & Cell Death - *Misfolding*)

Audio: **...to seed misfolding of normal alpha-synuclein proteins,... (sharp sound effect..?)**

Video: a-syn changes color to red while misfolding.

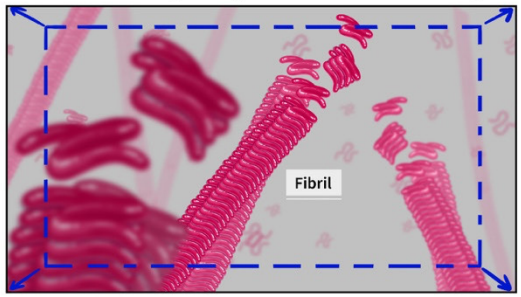
Scene - Shot: S5-3 (Toxicity & Cell Death - *Oligomerization*)



Audio: **...which subsequently aggregate into oligomers...**

Video: Repeat the oligomerization animation in S4-3.

Scene - Shot: S5-4 (Toxicity & Cell Death - *Fibrilization*)



Audio: **and fibrils.**

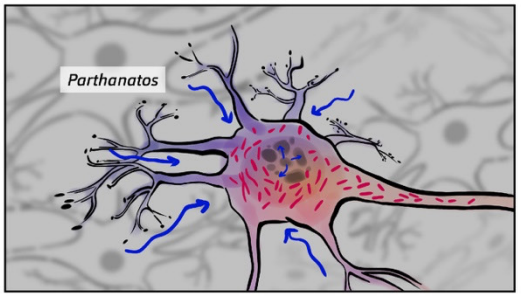
Video: Repeat the fibrilization animation in S4-4 - S4-6

Scene - Shot: S5-5 (Toxicity & Cell Death - *Toxicity*)

Audio: **The fibrils are toxic to neurons...**

Video: Focus on the 'hero' neuron with the fibrils inside; Normal state neuron (yellow/orange).

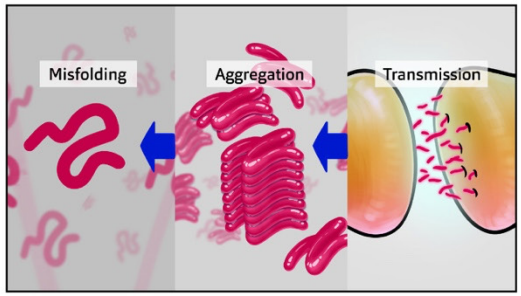
Scene - Shot: S5-6 (Toxicity & Cell Death - *Parthanatos*)



Audio: **...and eventually cause parthanatos, a type of cell death.**

Video: Depict cell morphology of Parthanatos: Loss of membrane integrity, nuclear DNA fragmentation (large) and cell shrinkage.

Scene - Shot: S6-1 (Neurodegeneration - *Repeated steps*)

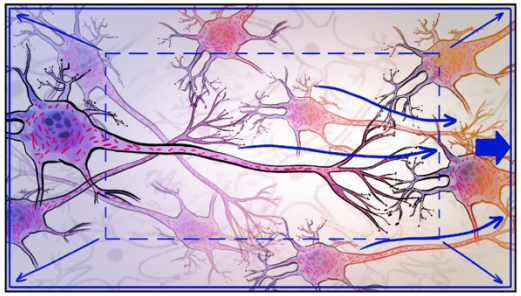


Audio: **After repeated steps of misfolding, aggregation, and transmission,...**

Video: [Aggregation] screen pushes [Misfolding] screen to left.
[Transmission] screen pushes [Aggregation] screen to left.

Figure 49. 3D Animation storyboard, page 8. Text not intended to be read.

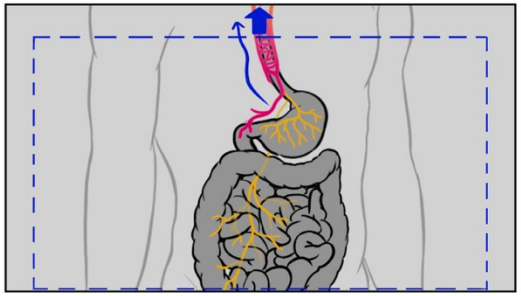
Scene - Shot: S6-2 (Neurodegeneration - *Cell death spreads*)



Audio: **...more and more neurons along the vagus nerve develop the toxic fibrils and die.**

Video: Cold & dark color (bluish purple) spreads from left to right; Pan right while zooming out.

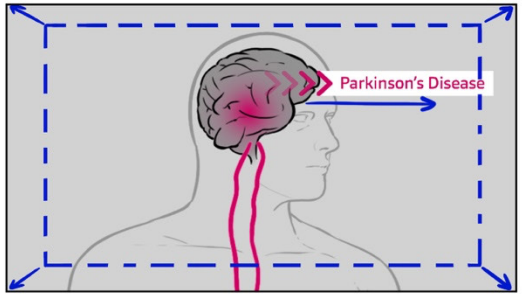
Scene - Shot: S6-3 (Neurodegeneration - *Propagation*)



Audio: **With time, the pathology propagates...**

Video: Magenta color spreads from the gut; Pan up.

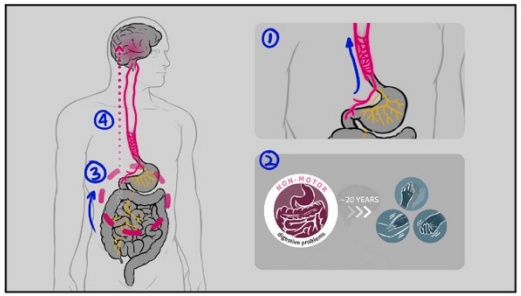
Scene - Shot: S6-4 (Neurodegeneration - *PD development*)



Audio: **...to reach the brain ultimately causing the symptoms associated with Parkinson's disease.**

Video: Magenta color reaches the brain; [Parkinson's Disease] fades in from left; Zoom out.

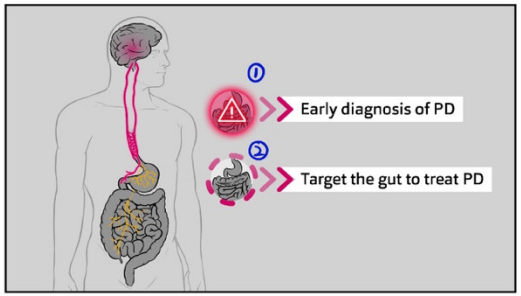
Scene - Shot: S7-1 (Conclusion - *Summary recapped*)



Audio: Although the cause of the initial misfolding in the gut remains unclear, the gut-to-brain spread of the pathogenic protein as well as the early onset of digestive problems suggest that Parkinson's may start in the gut.

Video: Move body to left; At "gut-to-brain", fade in 1 with magenta spreading upwards; At "early onset", fade in 2 with the arrows animated; Dashed circle moves around the gut at "originate"; Dotted arrow grows towards the brain.

Scene - Shot: S7-2 (Conclusion - *Future direction*)



Audio: **Further research may lead to early diagnosis of Parkinson's disease and pave the way for effective early interventions that target the gut instead of the brain.**

Video: For both 1 and 2, fade in the icon --> arrows --> then the texts.

Scene - Shot:

Audio:

Video:

Figure 50. 3D Animation storyboard, page 9. Text not intended to be read.

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Vita

Susie Yun was born in Cheongju, South Korea. She immigrated to Canada at a young age and spent most her time with her best friends since childhood: crayons, nature, and science books. During high school, her biology teacher found her notes filled with colored illustrations, and he strongly encouraged her to pursue a career in medical illustration. With this new passion in mind, Susie continued her science education in Western University where she received a BSc in Medical Sciences and Biology. During her time there, she saw beauty in the complexity of a human body: smaller units of cells and organs comprise the systems in the body, just like how tiny pencil strokes form shapes and altogether complete a whole picture.

Following graduation, Susie focused on improving her artistic abilities while helping people with autism learn and socialize, often using her own drawings as a communication tool. Now, as a graduate student in the Department of Art as Applied to Medicine, she is learning how to effectively bridge the gap between medicine and the target audience. In March of 2021, she was honored to be a recipient of the Vesalius Trust Research Grant for her thesis proposal. In the future, Susie hopes to communicate science better with minimally educated people, especially those with disabilities.